

What Williamsburg should say

Next week's economic summit is predictable — there will be a row about the US deficit. The prize to capture is a stronger link between technology and prosperity.

MEETINGS between heads of government such as that due to be held next week at Williamsburg, Virginia, are better remembered as opportunities for mutual misunderstanding than as occasions when the course of history is imaginatively redirected. So much is clear from what happened at the last Western economic summit at Versailles a year ago, when the issue of East-West trade in high technology was discussed, but too politely, with the result that within a few weeks the United States was at loggerheads with several Western European governments over the supply of equipment for the Soviet gas pipeline from Siberia. One obvious moral for Williamsburg is that plain speaking is in everybody's interests.

But what should the participants talk about? Rarely has the agenda for such a meeting been so closely determined by external events. After nearly a decade of economic troubles, the air is now full of the tentative cries of those who think that they have seen in the statistics evidence that the long recession may be coming to an end. The prophets of good cheer range from President Reagan to the chairmen of local chambers of commerce here and there, but their voices do not yet constitute a chorus — and, indeed, for every cheerful prophet there is another who says that economic recovery is still a long way off. Even the British Government, although in the middle of a general election, has been cautious, boasting that it has reduced the rate of inflation to 4 per cent a year but offering no specific promises about when and how the British economy will turn the corner. The French Government is further back along the painful road the British Government has been travelling, and is being forced by crude reality to abandon the high-spending plans on which it set its heart just over two years ago. Nobody should be surprised if President Mitterrand turns out to be the most truculent of those showing up at Williamsburg. But he will be only the most outspoken of those complaining that economic life everywhere would be better if only the United States Government conducted its affairs responsibly.

For the complaint is fair enough. The huge budget deficit the US Government plans to run at least until 1985 (when the next presidential election will have come and gone) is in itself a powerful obstacle to economic recovery. The explanation is straightforward. To pay its bills, the government has to borrow money from the markets — between now and the end of June, it will have to raise more than \$30,000 million on its way. If the lender is a bank, as is predominantly the case, the result is an increase of assets and thus an increased capacity to lend money to other people. If, on the other hand, the lender is a private person, he will have lent his money to the government and not to some productive enterprise — and can get his hands on it again only by borrowing from a bank against the security of his treasury certificates by paying a higher rate of interest. So industry is robbed of funds that it might have invested in economic expansion and there is also a risk that the increased supply of credit will increase the pace of inflation. The reality of that danger was plain last week, when the simplest measure of money supply in the United States increased by \$7,000 million and promptly stimulated an increase in the short-term interest rate. The whole process is thoroughly familiar. Governments that run huge budget deficits must choose between two evils — inflation and high interest rates. In accordance with the declarations of previous economic summits going back at least to 1975, the United States has plumped for high interest rates. But because the movement of money is now

thoroughly international, the consequence is that interest rates elsewhere must also be high. And so industrial recovery elsewhere is hampered by the Reagan Administration's budget policies.

That is the complaint. What response can President Reagan offer Williamsburg? To promise to do better in future, where the future means 1985, is the most that can be hoped for at this stage. The budget deficit stems from two independent sources — domestic spending, which neither side in Congress will tamper with in the year preceding a presidential election, and military spending on an unprecedented scale but on which President Reagan has staked his reputation (but may still be denied in Congress). But even a promise for the future would be worth having if it could be made in convincing terms. Once the financial markets were able to persuade themselves that interest rates would fall in the foreseeable future, they would busily set about discounting that development, banks outside the United States would be less eager to lend to the US Government, the value of the dollar would fall a little but economic activity would begin to pick up. The problem for President Reagan and his advisers, therefore, is to find a form of words that carries weight.

This central question is likely to dominate the proceedings at Williamsburg, as it should. Arguments about East-West trade in technology are unlikely to be contentious on this occasion. Arrangements intended to prevent the collapse of the international monetary system are similarly unlikely to take up much time, if only because the international banks appear to have learned to live with borrowers who repeatedly postpone the repayment of their loans. And Williamsburg is likely, like its predecessors, to utter yet another ringing call against protectionist restraints on world trade — a message that would carry more weight if only the governments participating were less zealous than they have become at trying to keep each others' commodities out. Only last month, the European Community (represented at Williamsburg) was boasting of a deal struck with Japan (also participating) whose effect will be to limit imports of Japanese video-recorders and other electronic goods to Western Europe, preferring that their own citizens should have to pay extra for these devices than that their own manufacturers should either be forced to be competitive or, better, persuaded into some field in which they can compete. And in the United States, the Commerce Committee in the House of Representatives is busily tidying up a bill designed to keep out imports of Japanese automobiles by means of a "non-tariff restraint on trade" along the lines of the bill that generated a fierce row almost exactly a year ago.

Against this background, the participants at Williamsburg will have to work hard to persuade their constituents that what they have to say is not mere window-dressing. They have one simple opportunity. These days, most governments in industrial states (and elsewhere too) have an ambition to use science and technology as a means of stimulating prosperity. The goal is plain but the means are not. On this occasion, the summit has had the wit to commission a study of opportunities for collaboration on scientific goals (see *Nature* 7 April, p.466) which includes a shopping list of sensible and sometimes imaginative basic research projects. The summit should give this document more than a mere casual endorsement. And it should go further, commissioning this time a study that will help at least some of the participants to make their technological dreams a little more realistic. □



No respite from politics

Attempts to lift environmental decisions above US politics are misguided.

THIS month marks the founding in the United States of a new scientific organization dedicated to "bridging the gap between the world of scientific discovery and the social and political centres which must act on these discoveries to conserve life". Dubbed the Collegium Ramazzini (after the seventeenth century Italian physician who pioneered occupational medicine), the organization hopes to bring government, labour, industry and scientists together to speak with a "single voice" on the regulation of occupational and environmental health. This goal is as noble as it is doomed. To be sure, the need to elevate the level of debate on these issues is great. The tactics of interested parties has seemed to drift more and more from science. Environmentalists continue to be guilty of over-reliance on emotional appeal and the public bandwagon (the environmental equivalent of the "disease-of-the-month club"), witness the storm over Love Canal, where — as inexcusable as Hooker Chemicals' actions were — no link between the waste dump and health problems among local residents has so far been proved.

Industry, on the other hand, is increasingly invoking the specious argument that regulation is improper without "scientific certainty" — a regulatory nirvana unattainable by mortal man, to judge by the industry definition of "certainty". It should not be necessary to have to point out what the consequences would have been of waiting for "certainty" before requiring chlorination of water, pasteurization of milk or, for that matter vaccination against smallpox.

Worse has been the emergence of nothing less than hired guns, experts who repeatedly appear as witnesses for one side or the other or, worse still, design experimental protocols that curiously yield results desired by their sponsors. This was embarrassingly apparent several years ago during the debate in the United States over regulation of emissions from diesel automobiles. All the tests run by the Environmental Protection Agency (EPA) — this was when EPA was still in the business of issuing regulations — showed a mutagenic hazard of diesel exhaust; all the tests run by General Motors (GM) showed the opposite. The difference lay almost completely in the experimental designs followed by the two groups. EPA researchers extracted their samples from diesel particulates using organic solvents, GM researchers used "simulated biological fluids", which they considered more realistic.

So one aim of the collegium — to be a "conscience" among scientists in this field — is apt; so too is the aim of publicizing the availability of information relating to critical occupational and environmental issues. But the notion that science will settle all disputes, that under the auspices of scientific guidance labour, industry and government will speak with one voice, is not only wrong but dangerous. The recent history of the National Academy of Sciences tells why.

The academy, like the collegium, was founded to provide high-level, impartial advice to policy-makers. But the academy's weight in prestige and impartiality has on at least two recent occasions been no match for the weight of political interests that it has crossed. When an academy report found the link between sulphur dioxide emissions and acidification of lakes convincing — and recommended an immediate cutback in acid deposition of 50 per cent — the White House declared the academy too biased to carry out a scientific review of acid rain in connection with the US-Canadian agreement. The White House empanelled its own hand-picked group of experts instead. And when academy members came down against a general recommendation that Americans should, across the board, reduce intake of cholesterol (and there is still no proof that dietary cholesterol affects serum cholesterol levels), consumer groups that had accepted the evils of dietary cholesterol and fat as gospel launched personal attacks on the committee members' motives and reputations.

The lesson is that when scientists enter the political arena, they are judged on political terms. It is simply not possible to declare

oneself impartial when making policy recommendations. The collegium should realize that rather than tempering conflict with their recommendations, as they hope, they are much more likely to be dragged into the conflict. A representative of Monsanto Chemicals, which is contributing to the support of the collegium, went so far as to say that the group's founding was at long last an acknowledgement that not only science but the interpretation of scientific results should be left to scientists — it is not for reporters or the public to interpret, and it is certainly not something to be debated on the front page of the *New York Times* or on television. In fact, he was completely wrong. Like it or not, regulation is a political matter, and scientists should not delude themselves that they, with their superior knowledge and superior impartiality, will somehow keep their hands clean as they delve into the sticky machinery of politics. Better that the process should be explicitly recognized as political — that what constitutes acceptable risk should be acknowledged as a matter for all of society to consider. The alternative is the very great danger that the genuine objectivity that science can bring to bear on society's problems will be tarnished as scientists are dragged into a political taking up of sides. □

Keith Joseph's mantle

British universities would profit from a radical suggestion from an outgoing education minister.

SIR Keith Joseph, the Secretary of State for Education and Science in the British Government at least until 10 June (the day after the election), has during the past two years enjoyed the bad luck of having to administer the contraction of British education, in the schools as well as higher education, and has also had a flair for digging pits into which he has promptly fallen. (The great row with the universities about the cost of retiring academics early, for example, remains unsettled — the universities suppose that the government will pay, the government that the University Grants Committee will find the money somewhere in its budget.) As a result, Sir Keith has been an unpopular minister. But he has also been, in his way, a kind of radical, willing to consider schemes for running this or that part of the educational system that differ sharply from present practice. One of the unfortunate consequences of the election is that it may never be known how Sir Keith would have handled a meeting arranged with half a dozen British universities to consider a proposal that some of them should become genuinely financially autonomous.

The meeting has been postponed, but the agenda has seen the light of day. The proposal is that a small number of British universities should, for an unspecified experimental period, be given an annual subvention by the government, linked with their present grant, and that they should afterwards be free to use their funds without external restraint. Sir Keith's agenda paper for the meeting that never happened suggests that in such a system, universities would be free to recruit as many students as they chose, that they should fix tuition fees without reference to external authority (but no doubt the market would have an influence) and that they should also become the channel by which this government helps with the cost of maintaining students, distributing those funds as they think best. In due course, the argument continues, universities would also be free to dispose of their capital assets.

Because of the legend that Sir Keith is universally to be distrusted, these proposals will be scoffed at in many British universities. They deserve a better hearing than that. For the kind of financial independence that Sir Keith has been flirting with is precisely the kind of independence needed to cultivate diversity within the British university system. Even if Sir Keith reappears after the election in some other guise, it is to be hoped that his successor will give them another airing. The objective of giving a handful of universities the freedom to make their own way in the world — and to be the victims of their own misjudgement — is admirable. What academic can object? □

Planetary science

US and Europe think big on future missions

Washington

PLANETARY scientists from Europe and the United States are about to ask their governments to pay for three joint space missions, costing between \$300 and \$800 million apiece, by the end of the century. They would include an ambitious double-orbiter and landing mission to Mars; a combined Saturn orbit and probe of Titan's atmosphere; and a "grand tour" rendezvous with a number of asteroids or comets.

The missions are being selected by a 12-member panel meeting under the aegis of the US National Academy of Sciences



and the European Science Foundation. The panel met last week in Washington and will publish details of the proposed missions in September after a final meeting in Munich. Both the European Space Agency (ESA) and the National Aeronautics and Space Administration (NASA) have attached observers to the panel.

By proposing an equal division of costs and benefits between NASA and ESA, the committee hopes to persuade the space agencies that they can afford to mount more ambitious expeditions than those now being considered. Last month, NASA released plans for a low-cost "core programme" of four planetary missions by the year 2000. The international group has crafted its proposals so that three of the NASA missions could be upgraded, with European participation, to produce a far greater scientific return.

If NASA were to adopt the core programme recommended by its Solar System exploration programme, the United States would mount flights to Venus, Mars, Titan and a comet or asteroid (see *Nature* 28 April, p.741). The NASA committee did, however, emphasize that several of the missions lent themselves to collaboration with international partners. The proposals now emerging from the international group are designed in such a way that the missions to Mars and Titan, and the

comet/asteroid mission, could be enlarged and mounted as joint ventures.

NASA's Mars mission, as envisaged in the core programme, would comprise a single orbiter designed to study the global surface composition of the planet and the role of water in the martian climate. The international group believes joint funding would enable this mission to be expanded to include two orbiters — one near and one more distant — with landings by surface penetration devices or, in an expensive variant, one or more surface rovers.

In the case of Titan, the NASA mission would use a modified Galileo probe together with a flyby or orbital satellite. With European collaboration, however, the mission would be upgraded to include an orbiter for Saturn and a separate probe of Titan's atmosphere. NASA's comet rendezvous and asteroid flyby, too, could be incorporated within a larger scheme under consideration by the international group, which entails a "grand tour" of several primitive planetary bodies and possibly a return of samples.

The international group chose the missions by looking for projects which were beyond the capabilities of the space agencies individually and which lent themselves to international partnerships.

A mission to the Moon was ruled out because of the number of missions already under consideration by the United States, the Soviet Union and Japan. In addition, the group believes that the important data needed from the Moon can be provided by a single orbiter, making a joint venture

difficult to organize. Jupiter was rejected because NASA already has a wealth of data about the planet and will have more after the Galileo flight. Both the United States and the Soviet Union are committed to Venus programmes, and a mission to Mercury was discounted because of the daunting technical problems raised by the planet's high temperature.

The missions to Mars and Saturn, on the other hand, could be devised so that the responsibilities of the two space agencies could be neatly split. Around Mars, one partner could operate a close-in orbiter studying the planet's geochemistry while the other operated a distant orbiter concentrating on the martian magnetic field and the shape of the planet. The Saturn mission could be split, with one partner mounting the probe of Titan's atmosphere and the other responsible for a Saturn orbiter.

Members of the international group expect to find it easier to win support from NASA than from ESA. The US members of the joint panel are led by Dr Eugene Levy from the University of Arizona, who helped to devise the NASA core programme. The international group's final recommendations are therefore likely to be carefully tailored to make the arguments for augmenting the core programme with European money extremely attractive.

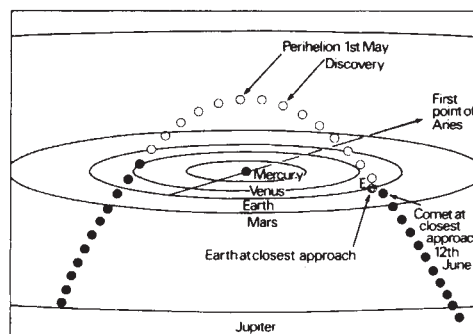
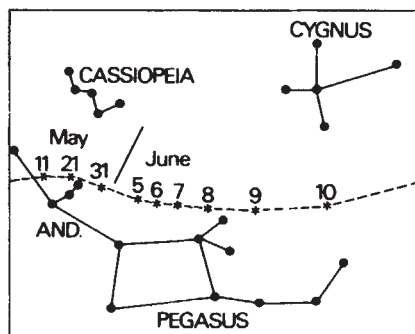
In ESA, however, planetary science appears to command a low priority, exemplified by the agency's recent decision to cancel the \$170 million Kepler mission, a plan for orbiting Mars, in favour of a more expensive infrared astronomy project. The joint group's European chairman, Professor Hugo Fichtig of the Max Planck Institute, hopes that European planetary scientists will be able to bypass or augment ESA's mandatory programme by enlarging the optional programme or persuading individual governments to pitch in with their own money.

Peter David

Another comet on the way

FOLLOWING hard on the recent comet IRAS-Araki-Alcock (1983d) comes Sugano-Saigusa-Fujikawa (1983e). Discovered on 8 May, the comet will pass closest to the Earth on 12 June, at a distance of 9.4 million km. Observers have detected a nucleus, coma and straight tail, and the comet may be just visible to the naked eye.

The figure on the right shows the orbit of the comet, open circles representing the portion above the ecliptic plane of the Solar system. On the left is displayed the orbit projected on the night sky, giving the positions at midnight on the dates indicated. (Diagrams supplied by N. Eaton and R. McCheyne, University of Leicester.) Philip Campbell



US research budget

Pork-barrel politics persists

Washington

THE science budget was subjected to some congressional politics-as-usual earlier this month when some last-minute manoeuvres by the leadership of the House of Representatives diverted \$5 million from the proposed National Center for Advanced Materials (NCAM) at the Lawrence Berkeley Laboratory in California to a new materials facility at Catholic University in Washington, DC. Outraged opponents had no sooner finished protesting — calling it pork-barrel politics and a subversion of Congress procedures — than a second amendment, giving \$5 million to Columbia University, was swiftly approved. One million dollars of this came out of funds earmarked for upgrading the Van de Graaf accelerators at Yale University and the University of Washington.

The amendments, offered on the House floor to the fiscal 1984 Energy Department authorization, were highly unusual. Representative James Sensenbrenner (Republican, Wisconsin) protested that the House Science and Technology Committee, which drafted the Energy Department authorization after months of hearings, had never even seen the two proposals. After learning of the proposal only the day before, Sensenbrenner said he discovered from Catholic University that there were no architectural plans for the facility (which will cost a total of \$13.9 million) and not even a written proposal for it. Several House staff members conceded that the action was “politically motivated” and a bit of “entrepreneurship” on the part of the universities.

The facility at Catholic University, the Vitreous State Laboratory, is spread over three buildings, and the new money would allow construction of a dedicated facility. The laboratory was originally supported by the Office of Naval Research to carry out basic studies on glassy materials and their applications, such as encapsulation of nuclear waste.

The Berkeley proposal, supported strongly by White House science adviser George Keyworth, had earlier this year been strongly criticized by materials scientists, who felt that it had been rushed into the President's budget request without adequate scientific review. The Science and Technology Committee had already cut \$5 million from the President's request for NCAM in response to this concern, before the bill reached the House floor.

The president of Catholic University, Father William Byron, acknowledged in an interview that the university thought of requesting funds for its laboratory only when it became aware in March of the administration's interest in materials research and of the problems NCAM was facing. “Certainly the procedure [scientific peer review and committee review] ought to be

followed”, he said, “but in this case it wasn't going to happen anyway.” He said he was confident that the work at the laboratory would stand up to any peer review, and he contended that the funding for Catholic University would not actually take money away from NCAM over its five-year construction period. “It's just a reallocation for the first year”, he said.

One House staff member cynically observed, however, that “it makes you almost nostalgic for the way NCAM got into the budget.”

Byron apparently approached the Speaker of the House, Representative Thomas “Tip” O'Neill, who then wrote a letter to the Science and Technology Committee chairman, Representative Don Fuqua (Democrat, Florida) on 28 April asking for his support. By that time the committee had already completed its mark-up of the authorization bill, however, and the only option remaining was an amendment on the House floor. Byron said that the Speaker had requested an add-on, not a re-direction of funding; but as it ended up,

one selling point of the amendment was that members were not being asked to increase the budget in voting for it.

The amendment was introduced by Representative Norman Mineta (Democrat, California), whose district is adjacent to Berkeley. “Everyone knew it was the Speaker's amendment”, said one House aide, who speculated that Mineta may have lent his endorsement in return for future assurances on NCAM.

The \$5 million diverted to the Columbia project, to be called the National Center for Chemical Research, was taken from the general authorizations for energy research, magnetic fusion and general science, and the specific authorization for the Yale and Washington accelerator upgrade. Yale officials, who were taken by surprise, said cutting their budget from \$8 million to \$7 million was not a “mortal wound” but would cause serious injury to their plans, and they would seek to preserve their budget when the bill comes before the Senate.

Both Catholic University and Columbia had retained the services of a Washington lobbying firm, Schlossberger-Cassidy and Associates, which has been aggressively recruiting university clients in the past year.

Stephen Budiansky

Export Administration Act

Congress backs scientific freedom

Washington

CONGRESS has taken an important first step towards redrafting the controversial Export Administration Act in a way that will make it harder in future for the government to prevent the publication of basic scientific information. But the amendment — accepted last week by the Foreign Affairs Committee of the House of Representatives — falls far short of the changes advocated last October by the National Academy of Sciences.

The act, which expires at the end of September, is one of the principal methods by which the Administration has attempted to halt what it describes as a haemorrhage of US technical and scientific secrets to the Soviet Union. It has also been used by the Department of Commerce to restrict the access of foreign scientists to militarily sensitive scientific information. In a report last year, the National Academy of Sciences recommended that unclassified information that was domestically available and not directly related to national security should be exempted from the act's formal licensing procedure.

A successor act submitted to Congress last month by the Commerce Department (see *Nature* 14 April, p.562) did not incorporate the academy's amendment. But last week the Foreign Affairs Committee voted in favour of an amendment inserting language in the preamble of the law which stresses the United States' commitment to free scientific exchange.

The two sentences, proposed by Repre-

sentative Lee Hamilton (Democrat, Indiana), say that it is the policy of the United States to sustain a “vigorous” scientific enterprise. “To do so requires protecting the ability of scientists and other scholars further to communicate their research findings by means of publication, teaching, conferences and other means of scholarly exchange”.

A third sentence which opponents of the act wanted inserted in the preamble, and which would have made it clear that the act's provisions could not apply to scientific communication, was dropped because too few members of the committee were prepared to give their support. But the truncated amendment may nevertheless have considerable importance. Representative Hamilton told the committee he intended to provide language for inclusion in the committee's report of the act which would spell out Congress's view that science depended on open publication.

Congressional reports on legislation have legal standing and are used by courts to determine findings. The committee has not yet received or endorsed the proposed language, but the report is likely to make it easier for scientists to challenge the application of the act to actions such as the submission of papers to international scientific meetings. One of the most controversial uses of the act was a decision by the Commerce Department in 1980 to prevent Soviet scientists from attending an American Vacuum Society symposium on magnetic bubble devices.

Peter David

French energy policy

Slump threatens nuclear freeze

FRANCE, the citadel of civil nuclear power in Western Europe, need not order another nuclear power station until 1987 at the earliest. That is the most startling of the conclusions of the "long-term energy group" of the planning ministry which put out an interim report on its work last week. The group's report says that France can get by for the next four years with its present complement of nuclear power stations (and the six under construction) unless economic growth exceeds 2.2 per cent this decade and 4.6 per cent in the 1990s. The chances of that happening are slim. Economic activity increased by 0.3 per cent in 1981, 2 per cent last year and is officially estimated at zero in 1983.

The nuclear industry is not the energy group's sole target. Gaz de France has signed agreements to buy in 1990 the equivalent of at least 29 million tonnes of oil a year as natural gas from Soviet, Algerian, Norwegian and other sources, but the group predicts an annual consumption of only 22 million tonnes of oil equivalent by then.

As the group sees it, the problem is twofold. The price of oil has fallen with respect to the dollar by 20 per cent in two years, undermining the incentive for large consumers to turn away from oil. And the economy has stopped growing, dashing hopes of a 5 per cent growth rate.

The planning group offers only one consolation — that the Mitterrand presidency's present policies of investment in innovation and research may begin to bear fruit after 1990.

What is to happen meanwhile? The report says that the energy industries can be supported only by a costly policy of minimum construction to keep them alive. For the nuclear industry, it recommends just one reactor a year — compared with three at present and as many as six in the heyday of nuclear expansion. The nuclear industry, naturally, does not consider this enough. Even at two a year, one company claims it would need to make 3,500 redundancies. Framatome, which builds the nuclear steam supply systems, has pronounced itself "anxious".

In the wake of the report, the great French effort in renewable energy (particularly biomass) and conservation may now be harder to defend than in the past. Indeed, some are even suggesting that the government should now provide incentives to consume energy, and that France should also export the stuff.

However, Michel Rolant, director of the energy-saving and alternatives body, Agence Française pour la Maîtrise de l'Énergie, has welcomed the report, and added that France must avoid "wasting investment" in nuclear plant, when the public financial situation and French indebtedness overseas are already so bad.

As for the government, the secretary of state has described the report as "illuminating".

Robert Walgate

● Pierre Mauroy, the French Prime Minister, announced figures last week that suggest his austerity programme — dubbed "rigueur" in France — is beginning to work. The overseas trade deficit fell from FF 6,500 million in March to FF 1,500 million in April. Exports rose 2 per cent. Special factors — such as unusually low oil imports — improved the picture, but the auguries are felt to be good, after a long period of despair. □

China stays friendly

Washington

CHINA seems to have decided not to allow political strains in its relations with the United States to damage the science and technology agreement signed by the two countries in 1979. A US delegation, led by presidential science adviser George Keyworth, returned from Peking (Beijing) last week with new agreements on nuclear physics, transportation, aeronautics and biomedical research under its belt.

The visit, a largely ceremonial annual event, came at a particularly bad moment diplomatically. The Chinese are still smarting after the defection to the United States of their tennis star, Hu Na. Relations have suffered again in recent weeks because of a legal dispute over the validity of a number of Hu-Guang railway bonds purchased by US citizens 80 years ago. Meanwhile, there has been little progress in the disagreement over Taiwan.

At a plenary session opening the Peking meeting, however, Fang Yi, former head of the Chinese Academy of Sciences and a signatory of the original agreement, was content to scold the United States for its curbs on technology transfer to China. In subsequent working meetings, said one US participant, the Chinese appeared to want business as usual.

Complaints about US restrictions on technology transfer were raised during the visit to China of Secretary of State George Shultz in February. On that occasion, Mr Shultz told the Chinese that the United States was restricting its technology curbs to those necessary for national security purposes. He claimed that the number of advanced technology export licences issued to the Chinese had trebled in three years, reaching 1,700 in 1982.

Measured by the number of exchanges between China and the United States, the 1979 accord has helped China more than the United States. There are more than 10,000 Chinese students and scholars in the United States, of whom 4,700 are government-supported exchange scholars. The number of Americans visiting China is around 500.

Peter David

Biotechnology

Biotech set to go public

WHO would pay \$40 million or a proportion thereof to own a corresponding share in a debt of \$10 million? Only a biotechnology investor. Many of that kind will be hammering on brokers' doors when Damon-Biotech, an enterprise based at the Massachusetts Institute of Technology (MIT) with ex-MIT president Jerome B. Wiesner on the board of directors, offers 2.4 million shares of its common stock to the public in the next few weeks.

Biotech, as it is commonly known, began life in 1978 with an ambition to produce and market monoclonal antibodies. Five years later, its chief claim on public attention seems to be a process for encapsulating cells in plastic porous membranes in such a way that they live, multiply and continue to secrete but are immune from damage.

The encapsulation process, patented and registered as Encapcel, described in the draft prospectus circulated on 28 April as an enabling technology, has certainly enabled the company to go public.

Since 1981, the company has had a contract with Hoffmann La Roche to produce gram quantities of antibodies against human alpha, beta and gamma interferon. This led in 1982 to a scale-up contract intended for producing kilogramme amounts of interferon antibodies. In the half-year to the end of February, Biotech claims sales of \$1.4 million, ahead of production and administration costs by \$350,000; research and interest charges led, however, to a loss of \$1.2 million.

The chief hope for the future is, however, the technique of encapsulation, described as a means by which animal cells may be cultured on a fermentation vat scale. Its invention is credited to Dr Franklin Kim of the Medical College of Virginia.

The technical trick is to immerse biological materials such as cells in a medium that can become a gel, to form small droplets of the medium and to make them gel, to coat the droplets with plastic and to reliquify the gel.

Although Biotech claims to have made a profit since and including 1980, overheads, research and interest have made its net loss fluctuate between \$1.4 and \$2.5 million a year. At 28 February 1983, the accumulated loss exceeds \$8 million, since when Damon has provided further sums to keep Biotech afloat.

\$10 million and now wants it back. It also holds 13 million shares which cost it \$130,000 and will own 70 per cent of the equity if the draft prospectus becomes reality. The offering at between \$16 and \$18 a share thus values Biotech at between \$400 and \$500 million — a little on the high side. □

London medical schools

Slow progress to mergers

MEDICAL education at the University of London is at last moving towards rationalization. Following a plan agreed some 18 months ago after thirteen years of intermittent deliberation, the university's medical schools are now changing with varying degrees of enthusiasm.

The reorganization has its roots in the 1960s, when in response to population decline in central London the National Health Service started diverting funds to the suburbs. Some teaching hospitals moved away from their historic central sites and so survived with their independence. Those that stayed are now facing radical change.

The need for change was given added urgency when cutbacks to recurrent grants announced in 1980, together with losses resulting from the government's overseas students policy (since partly recanted: see *Nature* 28 February, p. 643), left the general medical schools with budgets cut by 10 per cent. Months of wrangling finally resulted in a set of proposals from the university's Joint Planning Committee that were accepted by the court and senate at the end of 1981.

The university hoped to minimize duplication of teaching effort and to simplify bureaucracy by a series of mergers. Inevitably there were conflicts, with cherished traditions having to be weighed against more pragmatic considerations. Four major mergers were agreed, together with numerous administrative changes.

The first merger, announced in November 1980 and thus pre-empting the university's decision, was that of St Thomas's Hospital Medical School and Guy's Hospital Medical School, which declared their intention of forming a united school on their two sites south of the Thames. As the Joint Medical Schools of Guy's and St Thomas's, the institutions are now legally wed. Admissions procedures and preclinical curricula are being "harmonized", although they will remain formally distinct. A distracted administrator at St Thomas's points out that total integration is difficult with sites two miles apart. The joint schools have also taken on board the Institute of Dermatology, a refugee from the beleaguered British Postgraduate Medical Federation.

A new joint clinical school has also been established between Middlesex Hospital Medical School and University College's school, although here also preclinical entries are being kept separate "for the time being". The two sites, half a mile apart in Bloomsbury, run some joint departments and plan others, but the two schools remain separate legal entities.

Westminster Medical School and Charing Cross Hospital Medical School will be formally united in October 1984 and have already abandoned their separate curricula and constitutions. Dr P.A. Emerson, dean

of Westminster Medical School, feels the schools are complying with the spirit as well as the letter of the university's instructions. Westminster's preclinical students are already taught at its partner's site in Fulham.

The university also approved plans for a merger between the London Hospital Medical College and St Bartholomew's Hospital Medical College. The Royal Commission on Medical Education had originally suggested in 1968 that the two move their preclinical teaching onto a site adjacent to Queen Mary College, in east London. Negotiations continue. The matter is now being looked at by the City and East London Medical Education Group under Sir Frederick Dainton. Queen Mary College and the London — one of the poorest teaching hospitals — are keen to see the scheme go ahead. But some senior staff at St Bartholomew's, which has a substantial endowment income and an ancient City site, are less than enthusiastic. "No-one here really wants it to happen", one consultant said. The college has requested a careful study of the "complex considerations" but denies dragging its feet.

The University Grants Committee, originally keen to see a tangible result for all the agonizing, has asked for more detailed information before it will consider paying for the new buildings that will be necessary: it is apparently concerned that Queen Mary College will not itself have any surplus capacity. In the meantime some joint appointments have been made,

although teaching is largely separate.

The other schools remain separate, at least for the time being, but a working party under Sir Frank Hartley is studying one of the university's obvious embarrassments — the surplus capacity at St George's Hospital's site at Tooting in south London. Space for 800 science students and staff will be available from 1985, partly as a result of the decision by Chelsea College not to move there as had been expected. One of the proposals the working party will be considering is to move medically-related biological subjects from other colleges onto the site, a potentially uncomfortable arrangement.

Against the odds, the British Postgraduate Medical Federation has managed to survive, but has shed five of its smaller institutions to the general schools. The federation, principally concerned with specialist education, was badly hit by the British Government's policy on overseas students' fees, and many of the remaining institutes have lost staff. But the University Grants Committee has now relented a little and made "special arrangements". Hospitals have helped by shifting costs, but opinions are divided over the future role of specialist postgraduate institutions.

Doubts also remain over the future of the Institute of Basic Medical Sciences. The university has announced its intention of withdrawing support for the institute over a period of five years. The Royal College of Surgeons, which also supports the institute, is unhappy with the plan. The Royal College would be unable and unwilling to run the institute itself, and so is lobbying hard to keep the institute alive.

Tim Beardsley

French medical research

Soft budget saves hard science

INSERM, the French medical research council, seems to have staved off some of the worst of the budget cuts announced earlier this month (see *Nature* 19 May, p. 193). Laurent Fabius, minister of industry and research, had imposed cuts of 10 per cent (FF 43 million, or £3.7 million) in INSERM's equipment and building budget, and these cuts will stand; but the director of INSERM, Dr Philip Lazar, has now obtained FF 23 million from another source.

The source is the French social security fund, which has a separate existence from other items of the government budget, and is anyway favoured by the present socialist administration. The social security fund is divided into many sectors, and one of them is medical — *la caisse maladie*, with which Lazar has been able to negotiate a research contract worth FF 23 million.

However, INSERM researchers cannot yet be certain that they will be spared half the planned cuts. According to the nearly-completed contract, no research topic need be excluded but there must be an emphasis

on problems of interest to *la caisse*. So there would be a preference for health service subjects, such as the sociology and economics of health, the use and effects of innovations in health care and epidemiology. "Hard" biology, such as molecular biology, which receives considerable support from INSERM, would benefit where the work was related to medical problems.

This is the first such general contract agreed between INSERM and the social security fund, but probably not the last.

Meanwhile, Professor Pierre Papon, the director of the largest French research council, the Centre National de la Recherche Scientifique (CNRS), has told laboratory directors that the cuts suffered by his council (12.5 per cent, a little more than at INSERM) would be distributed in such a way that no laboratory would suffer more than a 13 per cent reduction of this year's running money. CNRS aims "to preserve the effort made over the past two years to give laboratories the means to do their work and to renew their equipment".

Robert Walgate

Vitamin C

Pauling backs wonder cures

Washington

CARRYING his crusade for vitamin C to the courtroom, Dr Linus Pauling appeared at a hearing on 11 May in San Francisco to defend a mail-order vitamin dealer charged with making false claims for his products.

The defendant, Oscar Falconi, has been selling vitamin C and other products under the name of the "Wholesale Nutrition Club", which does business out of two post-office boxes in California. His promotional literature includes such claims for vitamin C as "probably offers 100 per cent protection against bladder cancer", "cures (and prevents) urinary tract infections" and "prevents SIDS (sudden infant death syndrome)".

Falconi has also been offering a "Drug Rehabilitation Kit" consisting of three pounds of vitamin C powder and 265 multi-vitamin capsules together with instructions which assert that a regimen of the vitamins "allows you to kick alcohol, nicotine, caffeine, and Valium" as well as narcotics and other hard drugs. "If ever an addict is unconscious, and at death's door due to a drug overdose", the instructions add, "you must immediately find a doctor to slowly inject 30 to 40 grammes of our pure sodium ascorbate into the vein of the addict. If this is done in time, the addict will be out of trouble in minutes."



Pauling, who testified that "chronic subclinical scurvy" was widespread in the human population, appeared willing to defend even the most extreme of Falconi's claims. He cited Dr Ewan Cameron's disputed studies of cancer patients as evidence not only of vitamin C's effectiveness against cancer but also of its usefulness in curing drug addicts: cancer patients who were given 10 grammes of vitamin C per day, he said, stopped asking for morphine for their pain and suffered no withdrawal symptoms. Pauling also said that vitamin C has antibacterial and antiviral properties and acts synergistically with antibiotics.

Joining Pauling as a defence witness was Irwin Stone, a retired brewing chemist

(who when questioned about his credentials made several references to his honorary doctoral degrees from the Los Angeles College of Chiropractic and Donsbach University, a mail-order outfit; he in fact has no formal degree) whom Pauling credits for sparking his interest in vitamin C and health in 1966. Stone testified that "all clinical diseases have as their cause a lack of vitamin C".

The Postal Service, which brought the charges against Falconi under a statute prohibiting the use of the mail to obtain money by false representation, is seeking an administrative order to seize Falconi's incoming business mail. The Postal Service cited four specific products which it said Falconi misrepresented: the "Drug Rehabilitation Kit"; a vitamin-C test paper whose promotional literature included the exaggerated claims about vitamin C's benefits; the food

preservative BBT, which Falconi represents as a treatment for genital herpes; and a mixture of glycine and vitamin C for "detoxifying" coffee.

Dr Wallace Sampson of Stanford University and Dr Thomas Jukes of the University of California at Berkeley, both frequent critics of medical quackery, testified for the Postal Service.

According to Sandra McFeeley, who presented the Postal Service's case, Falconi has already agreed to the impounding of orders for the four disputed products while a decision is pending in the case; he has also agreed to refunds to those who ordered the products. McFeeley said the Postal Service is not seeking to put Falconi out of business nor accusing him of fraud, but only attempting to halt the misrepresentations. Falconi is, however, facing criminal charges of false advertising in the California courts.

A decision in the Postal Service's case is expected in three months.

Stephen Budiansky

Radioactive waste

US academy sets guidelines

Washington

THE search for a permanent underground repository for disposing of nuclear waste inched sluggishly forward last week with the publication of a major report by the National Academy of Sciences confirming that the technology is feasible and ready for testing. But the report — the first in which the academy has examined the overall waste disposal system — criticizes the way the Environmental Protection Agency (EPA) has tried to set safety criteria for a repository.

Under last year's Nuclear Waste Policy Act, the Department of Energy has until 1987 to choose a site for the first US repository, with tests using radioactive materials scheduled for 1990. The search has become increasingly urgent as a number of states have frozen the construction of nuclear power plants until an adequate national system for disposing of radioactive waste has been devised and is ready for use. The Department of Energy has started looking at nine sites in six states which may be suitable for a first geological repository.

The federal government has not yet set a "performance criterion" — an upper limit on radioactive leakage from such a repository. But the academy's report, prepared under the direction of Professor Thomas Pigford of the University of California, says EPA has approached the problem in the wrong way. Early performance criterion studies by EPA have examined the probable radiation dose to a total population within a 10,000-year period. The academy says a population-based calculation is neither useful nor meaningful, and proposes instead an upper limit on the lifetime dose to a maximally-

exposed individual.

In addition, the report argues against using a 10,000-year cut-off because only a "small fraction" of the radionuclides ultimately reaching the environment from a repository is expected to have been released during that time. The academy calculates future radiation dosages for as long as potentially important doses are predicted to occur. For the purposes of its own study, the academy selects 10^{-4} sieverts per year as the maximum acceptable average lifetime dose rate.

According to the academy, the first loadings of waste in the first repository, which should be completed before the end of the century, will be the products of spent fuel and defence waste that have been in storage for 30 years or more. The age of the material means that the heat generated by its decay will cause few problems, but newer waste added later will generate much higher temperatures and place greater stress on the packaging material. The report calls for new tests on the performance of borosilicate glass at high temperatures.

The academy's report discusses the merits of the various proposed sites, but makes no recommendations about which are most suitable. It says adequate sites are "probably" available in volcanic tuff but that they can be "most easily identified" in thick bedded salt deposits.

The Department of Energy has been holding public hearings at all the sites selected for study, and in most cases has encountered vigorous local opposition. At a hearing in Hereford, Texas, last week, department officials were told by angry residents that they refuse to accept "this nuclear trash".

Peter David

US animal welfare

Sweet reason emerges

Washington

THE National Academy of Sciences has begun once again its periodic revision of the official *Guide for the Care and Use of Laboratory Animals*, and last week held the first of three open hearings that were remarkable for their lack of rancour — an apparent legacy of some of the compromising that took place during last year's attempt to revise federal legislation governing the use of research animals.

The *Guide*, whose recommendations are binding upon recipients of National Institutes of Health (NIH) grants, is generally viewed as a model standard although the federal government has no general authority to enforce it.

At last week's hearing, animal welfare advocates struck a low key, focusing on a relatively narrow range of issues. Mrs Christine Stevens of the Animal Welfare Institute, a moderate group seeking reform rather than abolition of research involving animals, asked for a revision of rules on the exercise of animals (noting that dogs that have been previously housebroken especially need exercise outside their cages), and urged the academy committee to emphasize the need for researchers to ensure that euthanized animals are dead before disposing of them. She also noted the tendency of researchers to view the *Guide* as the "bible", and asked that cage-size recommendations be considered as minimum standards, not gospel.

Mr Howard Brown of the National Association of Life Science Industries, a trade group representing private testing

laboratories, concentrated on ventilation standards that the industry felt were not scientifically justified. But Brown also touched upon the issue that goes to the heart of the differences between researchers and welfare advocates when he made a plea for retaining the basic principle of "professional judgement" which is repeatedly invoked in the current *Guide*. The welfare advocates tend to the view expressed by Dr Donald Barnes of the National Antivivisection Society: "Stop guiding and start directing".

This issue is also central in the renewed efforts at legislative reform. Senator Robert Dole (Republican, Kansas) has introduced a bill (S.657) that would require as a matter of regulation that all institutions receiving federal research funds — and all federal agencies — should adhere to standards such as those in the *Guide*. The bill does answer two points raised last year by the research community, however: it drops last year's proposed requirement that institutions be accredited by a group such as the American Association for Accreditation of Laboratory Animal Care — which NIH said would cost \$500 million — and it specifically excludes any regulation of research design. It retains, however, the controversial requirement that institutions should establish animal care committees with at least one outside member to represent the interests of the humane community and that the committee should conduct semiannual inspections of all animal facilities.

Stephen Budiansky

Czechoslovak pollution

Past sins now whitewashed

TALK of pollution has at last become respectable in Czechoslovakia. Economic planners are concerned about the effects of pollution, including acid rain, on the country's forestry industry and industrial pollution, which five years ago could be discussed only in the underground press, has now reached the pages of even the Party daily *Rude Pravo*. Last month, the paper presented an alarming report on the pollution threat to Czechoslovakia's vital timber industry, noting that almost one-third of the forests of the Czech Socialist Republic were seriously affected by pollution.

According to what *Rude Pravo* described as "an analysis made by experts", the affected areas show between 10 and 70 per cent damage. In the worst hit areas, moreover, total clearance has been necessary, seriously disrupting the "extra-productive" or leisure use of the forests. In many areas, such as the Krusne mountains, the planting of strains of trees selected on the basis of expert results as most likely to resist adverse conditions has proved less "efficient" than originally estimated. Even these superior trees, it seems, cannot cope with present pollution levels.

The first official acknowledgment of the situation came at the end of last year, when it was noted that the state forestry industry would have to cut back on its annual cull of 11.7 million m² of timber to allow for the estimated annual loss of 600,000 to 800,000 m² to pollution damage. Last month's *Rude Pravo* article brought the whole matter into the open, identifying Czechoslovakia's lignite-fuelled thermal power stations as the major source of pollution and drawing attention to the secondary threat to the affected trees of insect damage, in particular by the fir sawfly.

In accordance with the usual principles of socialist realism, the full story has not been made public without the promise of remedial measures. Thus the state press agency CTK announced soon after publication of the *Rude Pravo* article that the Institute of Organic Chemistry of the Czechoslovak Academy of Sciences had developed a selective vanadium-based catalyst to deal with pollution by nitrogen oxides. The system is claimed to deal with 90 per cent of the nitrogen oxides in 120,000 m³ of gas emission an hour and is said already to have been installed in the North Bohemian Chemical Combine at Lovosice. CTK says that nitrogen oxide emission from the plant has been reduced by more than 4,000 tonnes per year and direct damage estimated at 7 million Czechoslovak crowns a year prevented.

Nothing has been said about remedial measures for sulphur dioxide pollution.

Vera Rich

No trips for Sakharov

ANDREI Sakharov's sixty-second birthday last Saturday was honoured in the United States by a special law which established it as "National Sakharov Day", and was commemorated by meetings and rallies in several European capitals. But the celebrations, honouring Sakharov's achievements in physics and human rights, were overshadowed by the decision of the Soviet authorities a few days earlier that Sakharov will not be allowed to leave the Soviet Union, because he still possesses sensitive defence-related information.

Since Sakharov's security clearance for classified information was withdrawn 15 years ago, the decision could be interpreted as casting an extremely doubtful light on the rate of Soviet military progress. In fact, it is clearly a formal excuse, designed to counter rumours during the past few weeks that Sakharov would be allowed to emigrate to take up a visiting lectureship at the University of Vienna.

Although the early reports cited anonymous and untraceable "diplomatic sources", on 8 May, Moscow radio's

Swedish service quoted the Minister of Justice, Vladimir Terebilov, as giving a broad hint that Sakharov could leave if he so wished. When Sakharov refused to go to Norway to receive his Nobel peace prize, said Terebilov, it was on his own initiative and "no one prevented him from travelling". Three days later, TASS stated in unequivocal terms that his former access to classified information excluded any possibility of even a brief foreign trip.

Sakharov himself, and his wife Elena Bonner, do not seem to have accepted the TASS announcement as final. During the past few days, Mrs Bonner has told foreign correspondents in Moscow that she fears for her husband's life, and called on the West to increase its pressure on his behalf. She said Sakharov now feels so isolated in his Gor'kii exile that he considers no further purpose can be served by his remaining voluntarily in the Soviet Union. The invitation which he would accept, however, is not the rumoured Vienna appointment, but one from the University of Oslo.

Vera Rich

ANZAAS Congress

Australia also seeks industrial innovation for prosperity

Perth, Western Australia

A NEW broom began to sweep the floor of Australian science at Australia's largest regular academic gathering here from 16 to 20 May — but only gently.

About 3,500 delegates attended the 33rd congress of the Australian and New Zealand Association for the Advancement of Science (ANZAAS), held in the spacious green environs of the University of Western Australia, the Western Australian Institute of Technology and Murdoch University. The balmy autumn weather may have tranquillized them into placid acceptance of the offerings of nearly a thousand papers and symposia, but there were only a few debates and, as usual, ANZAAS ended without a sense of progress in any direction.

Indeed, the sensation of this year's ANZAAS had nothing to do with science but with crime. Mr Douglas Meagher, senior counsel assisting the Royal Commission on the activities of the Federated Ship Painters and Dockers Union, issued at the very start of the congress a 213-page collection of revelations of the extent of organized crime throughout Australia, which at least allowed newspapers to add pointers to "other ANZAAS stories" otherwise buried on inside pages.

Between the planning of this congress over a year ago and last week, the economic and political climate of Australia had changed dramatically. The congress theme of "resources and responsibility" was more relevant to the erstwhile boom in exports of Australia's natural resources than to the current period of recession.

The new Labor Government has inherited from its Conservative predecessor an inflation rate of more than 11 per cent and an officially acknowledged unemployment rate of 9 per cent. The realization that economic recovery will be slow and painful is spreading, tempering expectations that the Labor election victory would lead to an early expansion of federal support for science.

Political priorities were highlighted by the failure of Mr Barry Jones, the new Minister for Science and Technology, to attend the ANZAAS meeting, a gathering normally embraced by the holder of his portfolio. Mr Jones was caught up in the crucial first parliamentary session of the new government, which brought down a cost-cutting mini-budget while the ANZAAS meeting was in progress. Mr Jones did take part, however, in one symposium by remote electronic means.

ANZAAS becomes more and more a non-scientific beast wearing the clothes of science. The social sciences and humanities

(including not only law and economics but musicology and women's studies) provided the great majority of the papers in the 34 specialized sections. In science proper, because presentation of a paper at an ANZAAS congress seldom leads to formal publication, participation and originality are inhibited, while the fact that ANZAAS papers are not refereed reduces their professional value.

Nonetheless, ANZAAS is not dying. Indeed, the organizers were exceptionally successful in attracting funds to bring 50 speakers of substance from overseas (more than at any previous congress) and in persuading hundreds of delegates to travel from the eastern states.

The new broom this year was that of encouraging technological innovation and development. The sweepers were led by Professor Ralph Slatyer, president of the congress, who is the new chairman of the Australian Science and Technology Council (having been appointed before the Malcolm Fraser government was defeated). A biologist at the Australian National University and a former Australian ambassador to UNESCO, he has a pivotal role in advising the Prime Minister on priorities for science and technology, including the programmes submitted by Mr Jones's own department.

In what would have been music to Mr Jones's distant ear, Professor Slatyer urged immediate action to improve Australia's economic performance and industrial competitiveness through accelerated technological innovation. He pitched his appeal for a major investment in research and education in terms of its potential effect on economic recovery and creation of jobs — a note tuned also to the ear of Prime Minister Bob Hawke, whose depth of interest in science and technology has yet to be publicly revealed.

Professor Slatyer illustrated his concern about Australia's capacity to lift itself out of recession by pointing out that less than 40 per cent of young Australians are enrolled full-time in the final years of secondary school, compared with more than 80 per cent in the United States and Japan. Further, since 1976, the numbers of students proceeding to full-time higher education have declined by 18 per cent, while on a *per capita* basis Australia has only about one-third as many engineers as Japan (24 as against 70 per 10,000 of population).

Slatyer also asserted that Australian science "appears to be as good as anywhere in the world" but that while Australian scientists produce some 2 per cent of the world's scientific knowledge, they are responsible for only 0.7 per cent of world

patents on which technological innovation is based. The isolation of scientific institutions from industry, little in-house research and development in industry (much of it foreign-owned) and cuts in funds for universities and for overseas contacts by scientists were cited by Professor Slatyer as priorities for correction.

One of the few speakers also to reflect the potential economic influences of a shift towards new technology was Dr Ken McCracken, chief of the Commonwealth Scientific and Industrial Research Organization's Division of Mineral Physics. He came down solidly in favour of a "Buy Australian" policy as the key element in an array of support mechanisms



Ralph Slatyer . . . urging more technological innovation

for selected industries, such as communications, small computers, nucleonics, word processing and electronic mail. Dr McCracken estimated a total expenditure within Australia after three years of about A\$300 million *per annum* and about 3,000 man-years of employment, before taking multiplier effects into account.

On Australian physics, formerly an area of notable innovation as in radio science, McCracken urged his fellows to speak up about the way government should help to turn the results of research into profit — or else they would find that funds for their research would dry up.

Although the communication of science to the public, one of the aims of every ANZAAS congress, was notable this year principally for its failure, the topic was examined in a special symposium. A survey of 31 editors of major newspapers, television and radio news services throughout Australia has revealed a generally unflattering image of scientists among these agenda-setters. The number of full-time science reporters in the Australian commercial media can be counted on one hand. There appeared to be agreement among those at this symposium that unless a better informed, more public profile for science and technology can be generated, the road to government-inspired initiatives in the future will be rocky indeed. **Peter Pockley**

Who goes to Oxbridge?

SIR — It is true ("Who goes to Oxbridge?", *Nature* 24 March, p.277) that Oxford and Cambridge selection procedures are being criticized for the apparently excessive number of students admitted from the independent fee-charging "public" schools, as compared with those from the state maintained schools. At Cambridge, for example, 48 per cent of those accepted in 1982 came from independent schools, attended by only about 5 per cent of their age group. This is the basis for the claim that the two ancient universities, as well as being admittedly "centres of excellence", are "also seats of privilege and are thus a baleful influence on British society as a whole", to quote from your article.

Oxford and Cambridge colleges have for many years been trying hard to select the best from among their applicants for admission as judged by purely academic standards and regardless of the schools they come from; and they try also to find promising candidates who may not have been well taught at school.

The Cambridge examination statistics are now published in a form from which some objective assessment may be made of any bias still remaining in the admissions system. Last year a special issue of the *Reporter* analysed the 1,930 men and 724 women who had completed their Part II (Final) Tripos examinations by, *inter alia*, the types of school from which they had been admitted, almost all in October 1979. Taking men and women together, 1,068 came from maintained schools and 862 from the independent sector, with a further 490 from "direct grant" schools and 234 "others", mostly from abroad. (The direct grant schools, which used to be a source of good candidates, were highly selective in their own admissions at the age of 11+, and in 1979 these were being phased out in favour of the comprehensive system of state education, which was to be entirely non-selective. Many of the more successful of the old direct grant schools chose to become independent.)

Looking simply at the results of candidates from maintained and independent sectors, 14.6 per cent of the 1,068 maintained school candidates were placed in Class I in the Part II examinations, significantly better than the 10.9 per cent of firsts for the independent school girls, compared with 8.8 per cent for the 329 from maintained schools. (Women at Cambridge, incidentally, consistently get fewer firsts than do men but, since they also get fewer thirds, the average performance of the two sexes is about the same.) Thus the maintained schools' advantage here is evidence for some bias against them in the admissions three years before, but the effect is not a large one: about 18 more of over 1,000 maintained school candidates finished in Class I than would have been expected had the results for the two types of school been

exactly the same.

Are relatively undeserving young ladies and gentlemen from public schools contriving to get places at the expense of worthier but less well taught candidates from the non-fee-paying sector? If so, this should show in their final examination results. Very few fail completely nowadays at Cambridge, with less than 2 per cent appearing under the two headings of "Allowances" towards the Ordinary (not Honours) degree, and of "Others" which include withdrawals for non-academic reasons as well as outright failures. But anyone who can do no better than Class III should probably also be counted as an admissions mistake, having got the place in preference to someone else who ought to have done better. Doing the same analysis as for Class I on the Third Class results, plus the few Allowances and Others, shows that 9.5 per cent of those admitted from maintained schools appear in this relatively unsatisfactory category, as compared with 9.2 per cent from independent schools, the difference not being statistically significant.

So if it can be agreed that Cambridge ought to be selecting the academically best of those who apply for admission, it seems that any bias that can be detected in these statistics is really very small indeed. Critics might be hard put to suggest a way of achieving fairer results.

C.B. GOODHART

*Gonville and Caius College,
Cambridge, UK*

SIR — I have read your editorial on Oxbridge (*Nature* 24 March, p.277), and I agree with you that the danger is that the universities will trim their sails to the wind so that they damage themselves, and by extension British education as a whole.

But that is about as far as my agreement goes. It seems to me that you propose a recipe for the disaster you seek to avoid.

D.A.H. TAYLOR

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and Applied Chemistry,
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Bedford College

SIR — May I take you up on an inaccuracy in the article "Merger, merger everywhere" (*Nature* 31 March, p.369). Bedford College is not only transferring its science teaching to Royal Holloway College but is merging completely with Royal Holloway to form a new college of about 3,000 students in the faculties of arts, music and science including the social sciences. We hope that through this merger we shall achieve a college which is strong and vigorous in both teaching and research.

L.P. TURNBULL
(Registrar)

*Bedford College,
London NW1, UK*

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SIR — I have recently completed a book, *Quaternary Paleoclimatology*, and have thereby become aware of the problems authors now face over gaining permission to reproduce copyrighted material. To reproduce a figure directly you must first write to the copyright holder, generally the journal or publisher. This would seem to be straightforward enough; however, most of these organizations require that you contact the authors and obtain their permission also. What, then, is the purpose of journals requiring that authors sign over copyright permission to them?

Furthermore, some journals (such as *Science*) require that a fee be paid for each figure permission granted. Presumably the authors whom the journal requires that you contact could do the same. In my case, some have requested copies of the book when published. Although each fee may be small, in a large book fees may easily mount up to \$1,000 or more, and this can only result in a higher price for the book. Other, more enlightened, organizations give *carte blanche* permission providing adequate citations are given, thereby avoiding masses of paperwork and accounting. In my case alone correspondence has involved literally hundreds of letters back and forth and collectively has probably consumed hundreds of man-hours and many thousands of dollars in secretarial costs and so on. It has certainly been the most frustrating aspect of writing the book. Surely it is in the best interests of the scientific community for publishers to state prominently in each journal that permission to reproduce one or two figures per article is automatically granted providing proper credit is given. This would aid communication, save time and money and avoid a great deal of aggravation for authors.

RAYMOND S. BRADLEY

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How to do physics by numbers

Computers, widely used for solving intractable problems numerically, seem also to stimulate the imagination. Two recent examples confirm.

THE simple truth that most problems in physics are not exactly soluble is widely appreciated. Generally speaking, there are two strategies for dealing with these awkward circumstances. One is to substitute for the real problem an idealization of it that can be tackled analytically, as when the motion of the Moon around the Earth is represented by one point mass travelling about another. The alternative is to fall back on mathematical approximations and the numerical methods that go with them, nicely exemplified by the complications of constructing accurate expressions for the motion of the real aspherical Moon around the aspherical (and inelastic) Earth in the presence of the Sun.

Now, with the arrival of computers, not merely is the position of the Moon easily and accurately calculable, but it is even possible to delegate to computer codes the tedious algebra entailed in working through the mathematical approximations. The consequence of such developments is not merely that numerical solutions have become more realistic, but that people's imaginations have been stimulated.

Quantum mechanics is an obvious field for computational innovation, if only because so few problems can be solved exactly. Those teaching the subject are hamstrung by the rarity of exact solutions of the Schrödinger equation. Similar difficulties crop up throughout the field, with the result that a few months ago A.H. Cooke thought it worth publishing a catalogue of the solutions of Dirac's equations (*Proc. R. Soc. A* **383**, 247–278; 1982).

In such circumstances, numerical methods of solution flourish, but even so it is something of a surprise that Carl M. Bender (from Washington University, St Louis) and David H. Sharp (from Los Alamos) should be able to announce the application of the method of finite elements, much loved by engineers, to the solution of equations describing operator fields (*Phys. Rev. Lett.* **50**, 1535; 1983).

The method of finite elements, essentially what R. V. Southwell used to call the "relaxation" method, is simple enough when applied to the solution of a differential equation. For a first-order equation containing only first differential coefficients of some quantity, for example, it is sufficient to divide the range of the independent variable or variables into a large number of pieces of equal length and to represent the dependent variable whose

solution is sought by a series of linear functions, one in each subdivision of the range. Requiring that the slope in each successive subdivision of the range should be that specified by the original differential equation then yields a string of numerical relationships between the values of the unknown quantity at the two ends of each subdivision of the range.

But what happens if the variables are not ordinary functions but, rather, operators in the sense of quantum mechanics? This is the starting point for the exercise that Bender and Sharp have undertaken. By way of illustration they take the example of a one-dimensional system, defined by a hamiltonian of the form $p^2/2 + V(q)$ where p and q are respectively the momentum and position variables and $V(q)$ is some potential function. Those bent on finding the stationary states of such a system know well enough how to proceed — convert the hamiltonian into a differential operator by the usual rules and look for wave functions corresponding to eigenstates. But what if time-dependence is the essence of the problem, so that both p and q are operators which are functions of the time t ? The quantum mechanical analogues of Hamilton's classical equations are then $dp(t)/dt = f(q(t))$ and $dq(t)/dt = p(t)$ where the function f is $-dV/dq$.

So, in practice, it is feasible to think of solving this pair of simultaneous first-order equations for p and q by the method of finite elements, chopping up the range of time into suitably small elements and starting with suitable initial values for the two operators. The snag is that such an interaction will yield nonsense unless the operators p and q , conjugate momentum and position as they are, satisfy the commutation relations $q(t)p(t) - p(t)q(t) = i$ (in units such that $\hbar/2\pi = 1$) at all points bounding subdivisions of the time range. What Bender and Sharp have done is to show that the commutation relations are indeed preserved by the iteration process and then, by heroic algebra, that the corresponding conditions survive in the iterative time-evolution of a simple scalar field. The chief value of their method will be to sharpen physical intuition.

Much the same may be true of the technique described by Michael Creutz (from Brookhaven National Laboratory) for calculating the partition function of some physical system (*Phys. Rev. Lett.* **50**, 1411; 1983). Since the time of Willard Gibbs, statistical mechanics has been based on the

argument that the entropy of a system can be calculated from the number of configurations compatible with such external constraints as there may be, for example, its fixed energy.

The practice of statistical mechanics is in essence the calculation of the numbers of configurations compatible with this or that constraint, but with the advent of computers people have recognized that many intractable jobs can be done, at least numerically, by machine. One way of tackling such problems is by a version of the Monte Carlo method — set up a computer model of the microscopic components of a system, let the system evolve in the course of time but randomly, program the machine to recognize distinct configurations . . . and simply let it run. For then, by Poincaré's ergodic theorem, the system will eventually run through all possible configurations compatible with the constraints. Problems such as that of a lattice whose vertices carry entities which interact with their neighbours are conveniently dealt with, and have the added attraction that they may also yield solutions to problems in the gauge theory of particle fields (see *Nature* **299**, 675; 1982).

Creutz's amusing innovation in this process is essentially a trick for simplifying the imposition of external constraints. He postulates (and builds into his programs) a microscopic "demon" that wanders from place to place, using a portable stock of energy to change the state of some microscopic element. The rules of the game are that the demon puts in his "sack" such energy as it manages to collect, but is not able to change the state of a microscopic component if the result would that his own stock of energy was exhausted.

One advantage of this arrangement is its simplicity. Programming demons is child's play. Moreover, the mere existence of a demon carrying part of the energy of the system turns that into what Gibbs called a microcanonical ensemble — a system large enough for thermodynamic quantities such as temperature to be defined, but which is nevertheless part of some larger system. The result is that the average energy carried by the demon can be directly related to the temperature. Creutz's own interest in gauge theories leads him to offer illustrations of the working of his technique in the context of SU(2) theory, but he also points to the intriguing possibility that a demon could be programmed to conserve more interesting quantities.

John Maddox

High-energy physics

Closing in on the distant Z^0

from Frank Close

THE electrically charged W particle — the carrier of the weak force — was discovered in January at CERN. Physicists there are now searching for its electrically neutral counterpart — the Z — and according to preliminary reports (see *Nature*, 19 May, page 193) may have found one example.

But even before this latest event, the effects of the existence of the particle may have been detected 'from afar' in recent experiments at PETRA, Hamburg where electrons and positrons are annihilated (reviewed by M. Davier at the International Conference on High Energy Physics, Paris, 1982). These experiments are not powerful enough to produce the Z particle physically in the laboratory (the Z is predicted to weigh of order 90 proton masses), so for the moment, at least, detection of its presence must be by more subtle indirect means.

The first results from the analogous machine PEP in the USA have now been reported (E. Fernandez *et al.* *Phys. Rev. Lett.* **50**, 1238; 1983). The experiment was performed using electrons and positrons of lower energy than at Hamburg. As a result it is possible to isolate the energy dependence of effects that arise from the Z particle's Pickwickian presence.

In many ways the Z particle is like a massive version of the familiar photon. The photon plays an essential part in transmitting electromagnetic forces between electrically charged particles. The Z particle by analogy also can be transmitted between charged particles and thereby cause them to feel an extra force, as well as the electromagnetic one. This extra force is an example of the weak interaction, whose best known distinguishing property is that of violating mirror symmetry (parity violation).

This parity violation is a tell-tale signature of the weak force at work and so physicists have looked for examples of processes which at first sight are purely electromagnetic but on closer examination reveal evidence for parity violation. Indeed, the model of Weinberg and Salam uniting electromagnetic and weak forces requires the Z particle to exist and transmit the weak force with a characteristic magnitude of parity violation. This model also predicts manifestations of the weak force that conserve parity but generate other observable effects on particle interactions; the electron-positron experiments are examples of parity-conserving interactions.

Before describing the PETRA and DEP experiments it may be of interest to review the history of these phenomena, and the original experiments that concentrated on parity-violating effects. The first clues that

the Weinberg-Salam model was on the right lines came a decade ago with the discovery of the weak-neutral interaction between neutrinos and matter, in particular the elastic scattering of neutrinos on nuclei. This process was something quite new: electrically neutral neutrinos do not directly experience electromagnetic interactions, and when they participate in weak interactions it had seemed that electrons were necessarily produced in consequence — either in partnership, as in familiar β -decay; or through the transformation of the neutrinos as they scattered from the target. The discovery that neutrinos could scatter without changing their identity was not only a dramatic new observation of a natural phenomenon, it was also a fulfilment of one of the predictions of the Weinberg-Salam model. It was the discovery of the weak-neutral interaction at CERN, perhaps more than any other, that convinced physicists that the Weinberg-Salam model may indeed have something to do with nature.

That first observation was of the new weak interaction operating on its own. In 1978 a beautiful experiment showed this new weak force at work in competition with the familiar electromagnetic force (*Nature* **274**, 11). At Stanford University in California beams of electrons were prepared so that the electrons were corkscrewing in either a left-handed or right-handed sense ('polarized electrons'). These high-energy electrons were then scattered from a deuterium nucleus and their distribution after the scattering was found to be inconsistent with that expected if the electromagnetic force alone had acted on them. A small violation of mirror-symmetry was detected, of precisely the amount expected if the weak force was also acting in the electron scattering.

Instead of scattering electrons from nuclei one can annihilate them with their antimatter equivalent — positrons (e^+). After the annihilation new varieties of matter and antimatter can appear, such as quarks and antiquarks or muons and antimuons ($\mu^-\mu^+$). These are all well known examples of electromagnetism at work.

However, as above, the Weinberg-Salam model predicts that the weak force will also contribute here. When electrons and positrons mutually annihilate at low energies the effect of the weak force is minimal, being totally dominated by the familiar electromagnetic interaction. But if the energy of the electron-positron annihilation is high enough, the weak force should become less feeble. Indeed, if Weinberg and Salam are right and the weak and the electromagnetic forces are truly unified, then by the order of 100 GeV an-

nihilation energy, the Z particle should be physically produced in the laboratory as the weak force and electromagnetic force become equally important.

There is as yet no electron-positron facility powerful enough to produce such a massive Z particle, though protons and antiprotons are being annihilated at CERN with enough ferocity to encourage hopes that recent claims to have detected the Z may soon be substantiated. The highest electron-positron annihilation energy currently available is of the order of 40 GeV — far short of that required to produce the Z but large enough for weak-interaction effects to become sizeable.

How are these effects manifested? The electrons and positrons collide head-on along some direction that is normal to an imaginary plane. This plane divides space into hemispheres, and we shall call the one the initial positron was heading for the 'forward' hemisphere. If the electron-positron annihilation were controlled only by the electromagnetic interaction, then processes that produce μ^- and μ^+ would yield the μ^+ in the forward hemisphere marginally more often than in the backward. (Momentum is balanced by the μ^- emerging in the other hemisphere.) If the weak interaction also plays a part, as in the Weinberg-Salam model, the μ^+ should emerge more often in the backward hemisphere.

This backward excess is precisely what the experiments show and the mismatch between the forward and backward hemispheres grows as the energy grows — as the theory predicts it should. The asymmetry cannot grow for ever or you would eventually get a nonsensical result — more than 100 per cent would come out backwards; and this paradoxical result is prevented, according to theory, by the physical production of a Z in experiments at higher energies. At this point, the asymmetry would cease to grow.

The change in the growth of asymmetry with energy does not happen abruptly. By studying the way that the asymmetry is growing even at currently available energies, it may be possible to tell by extrapolation at what energy it will cut off and the Z thus appear. The latest results (*Phys. Rev. Lett.* **50**, 1138) were from an experiment where the total annihilation energy was 29 GeV. Combined with data from Hamburg which are taken at energies of up to 35 GeV, one can by extrapolation infer already that the mass of the Z is greater than 50 GeV and probably less than infinity. The theorists predict that the Z should weigh of order 90 GeV, and this is supported by the observation of its partner, the electrically charged W , at 80 GeV — which is consistent with the theory. The quantitative form of the asymmetry — not just how many come out in the forward compared with backward half, but also the distributions of the muons throughout space — are in good agreement with that expected if the Z indeed weighs around 90

GeV.

Some interesting extensions have been made to these experiments. One could, for example, enhance the effect of the weak interactions by reducing the 'unwanted' electromagnetic contribution. The strength of the electromagnetic contribution is proportional among other things to the square of the electrical charge of the produced particles, in this case muons. But it is possible instead of studying muon-antimuon production to study quark-antiquark production; because quarks have fractional charges, this reduces the importance of the electromagnetic contribution while the weak contribution stays essentially unaltered. As a result, the weak signal grows relative to the electromagnetic noise. The asymmetry should increase by as much as three times that in the muon-production experiments. The catch is that one cannot detect free quarks — they emerge in clusters, the familiar π , K or charmed particles. However, if you detect a fast charmed meson you are to a good approximation seeing the effects due to the

charmed quark itself. Asymmetries are again observed of the magnitude and character predicted by the theory.

These are beautiful high-precision experiments with large complicated apparatus. They have detected and quantified one of nature's most subtle effects. It will, of course, be most interesting in the coming decades to see what happens when the electrons and positrons annihilate at energies that are high enough to produce the Z physically in the laboratory — by which point the parity-violating weak force and the good old electromagnetic force will be in manifest competition.

I am indebted to Dr R. Marshall (JADE) and Dr D. Saxon (TASSO) for information about the Hamburg experiments and impressing upon me the physical significance of some of these phenomena. □

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Gene regulation

Enhancer elements in immunoglobulin genes

from Michael A. Boss

EXPERIMENTS designed to explore the control of antibody production have revealed for the first time the existence of enhancer sequences in eukaryotic genes. Enhancers, until now identified only in viruses, are short regulatory sequences that increase the rate of transcription from many eukaryotic promoters, irrespective of their orientation or — within reason — their distance from the promoter¹. Although the enhancer elements of different viruses all have a similar core element², their sequences may otherwise vary considerably. This variation may determine in part the species- and tissue-specificity of the virus, and is of particular interest because it may thus reflect the normal mechanisms of tissue-specific control of gene expression. Such a normal mechanism has now been discovered in the regulation of immunoglobulin genes, and was described by several speakers at a recent meeting*.

Two neighbouring but independent groups — those of David Baltimore and Susumu Tonegawa, both at MIT — presented important new data on the expression of cloned immunoglobulin genes introduced into myeloma cells. Immunoglobulin gene expression has attracted particular attention because of the unique DNA rearrangements that accompany it. The complete gene for each chain of the immunoglobulin molecule is

assembled in the course of lymphocyte differentiation by the translocation of one of several hundred variable-region gene segments (V segments) to the site of the constant-region gene segment (C segment). Each V segment has its own promoter but is transcriptionally silent in its original position. The C segment has a low level of constitutive activity which is increased by the rearrangement that juxtaposes it with a V segment.

The basis of this activation has recently been the focus of even more than usual interest because of suggestions that the same mechanism may account for the activation of the *myc* cellular oncogene, which undergoes aberrant translocation into the immunoglobulin locus in some tumours³. Until recently however, attempts to explore the mechanism by transfecting cells with cloned immunoglobulin genes have met with limited success⁴. It now seems possible that this is because the transfection experiments were done with non-lymphoid cells.

C. Queen, now at NCI, and D. Baltimore reported the effects on transcriptional control of alterations *in vitro* to a complete immunoglobulin α light-chain gene. The basic construct consisted of a plasmid containing an intact α gene with a simian virus 40 (SV40) origin of replication and a polyoma early region containing the promoter. When myeloma cells were transfected with the plasmid, transient α -gene expression could be

demonstrated (by protection of a 5' probe from digestion with S₁ nuclease). However, when the 3' part of the gene, including part of the J_H-C_H intron and the whole of the C_H segment, was removed and replaced with a SV40 poly(A) site, the remaining α sequence was no longer expressed in myeloma cells.

The part of the J_H-C_H intron that had been removed contains a site that in active α genes is hypersensitive to digestion by DNase^{5,6}. Such sites are usually found near the transcriptional initiation site at the 5' end of the gene⁷ and within enhancer elements (ref. 8 and J. Jongstra and P. Chambon, CNRS). The site in the α intron coincides with a region conserved between mouse and human genes⁹ and has sequence homology to the 72-base pair (bp) SV40 enhancer repeat¹⁰. Queen and Baltimore suggest that this region, which is deleted in their variant α construct, is indeed acting as an enhancer, although it is not yet known whether it operates regardless of orientation and distance from the promoter, and thus has the defining properties of a true enhancer.

S. Gillies, with S. Tonegawa, presented more direct evidence for the presence of an enhancer in the immunoglobulin heavy-chain gene. They transfected myeloma cells with a functionally rearranged γ 2b heavy-chain gene on a pSV2 gpt-based plasmid, and derived from them cell lines expressing γ 2b protein.

A deletion downstream of the *Eco*RI site in the J_H-C_H intron resulted in only a slight reduction in levels of both RNA and protein; but a deletion that spanned the *Eco*RI site resulted in complete loss of transcription. In further experiments, the DNA surrounding the *Eco*RI site was isolated on a 1-kilobase (kb) *Xba* fragment and reinserted either at its original position but in the opposite orientation, or 0.8 kb upstream of the promoter in both orientations. With all three constructs protein expression was restored to qualitatively similar levels, as shown by immunofluorescence studies. The enhancer element was moreover found to have sequence homology to the core sequence of known enhancer elements. Thus a sequence with all the properties of a true enhancer is located upstream of the immunoglobulin heavy-chain switch region¹¹. This is the position of maximum economy, since the sequence need then be present only once for control of all heavy-chain isotypes.

Although enhancers may operate over considerable distances, there must be limits to the range of the immunoglobulin-gene enhancer elements, because only the V segment that has become rearranged to the site of the C segment is activated. The several hundred other V segments upstream of the rearranged segment remain silent¹². There is in fact suggestive evidence that enhancers function effectively only over short distances¹³: Picard and Schaffner have shown that an immunoglobulin λ gene in non-lymphoid cells can be driven by SV40

*The Cetus-UCLA meeting on 'Gene Expression' was held at Park City, Utah on 26 March-2 April 1983.

enhancer elements only when there is less than 1 kb of 5'-flanking sequence between the cap site and the SV40 repeats.

Tissue-specificity in the action of immunoglobulin gene enhancers may explain some earlier failures of expression in transfected cells. The complete mouse κ -gene construct of Queen and Baltimore is expressed only at low levels in mouse 3T3 fibroblasts. Similarly a different mouse κ gene was not expressed at all in mouse L cells under the control of its own promoter¹⁴, and a mouse λ gene was expressed incorrectly in mouse fibroblasts and human or monkey cells¹³.

There is considerable interest in knowing whether enhancers are common elements in eukaryotic genomes. Human papovavirus BKV 68-bp repeats that have been shown to enhance the chloramphenicol acetyltransferase (CAT) gene in HeLa cells were accordingly used as a probe to screen a human genomic library (N. Rosenthal and G. Khoury, NCI). A unique clone was isolated which had about 100 20-21-bp repeats in tandem, with a 'core' sequence similar to that of the BKV repeats. This human fragment was found to function as a low-level enhancer in the CAT assay (although it is not known whether it has any activity *in vivo*). It remains to be determined whether the BKV-related and immunoglobulin enhancers are rare elements in eukaryotic genomes, or represent families of related structures with common core elements but different flanking sequences responsible for tissue-specific gene expression of a large number of proteins.

In the meantime, the immunoglobulin gene enhancer has implications for the activation of cellular *myc* genes translocated to the immunoglobulin heavy-chain locus. In human tumours with such translocations, the *c-myc* gene seems to be orientated in the opposite transcriptional direction from the immunoglobulin genes. If *c-myc* activation were due to the immunoglobulin enhancer, clearly the orientation of the oncogene would not matter. □

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Space Shuttle

Spacecraft that glows in the night

from Michael J. Rycroft

THE Space Shuttle has very considerable potential for carrying large instruments into space. Many of the instruments will view the Earth, or the cosmos, in the visible and/or infrared (IR) parts of the electromagnetic spectrum. Since optical observations made above the atmosphere are not subject to absorption or scintillation (twinkling), which always limit the quality of observations made from Earth, it is expected that high-quality results should be obtained from the Space Shuttle. However, a recent discovery that the Space Shuttle itself glows may well limit the quality of observations made from this platform in space.

The stunning colour photograph on the front cover of the February 1983 issue of *Geophysical Research Letters* is a night-time view taken from the aft flight-deck window during the third Space Shuttle mission of March 1982. From an altitude of 240 km, the foreground is illuminated by the filament of an electron emitter which is used to investigate the potential of the Shuttle with respect to the potential of the surrounding ionospheric plasma. In the middle distance there is an orange or pinkish glow just off one side of the orbiter's tail, but not off the other. And in the background is seen a greenish band of airglow emitted from the limb of the Earth at an altitude of about 100 km over the southern polar regions, and many stars.

Not only has this glow been noted from the Space Shuttle, it has also been observed from rockets, NASA's Atmospheric Explorer-C and -E satellites, and in the laboratory. For the fourth Shuttle mission, a simple spectrographical experiment was devised to investigate the glow further, using a conventional camera equipped with an objective transmission grating, again viewing from the aft flight-deck window.

Five consecutive papers in *Geophysical Research Letters*¹⁻⁵ are devoted to a discussion of the properties, origin and consequences of the spacecraft glow. The glow radiation is emitted over a continuum, and not in discrete spectral lines, in the visible region of the spectrum, and is strongest at the longer wavelengths, particularly beyond 0.63 μm . It is strongest when the Shuttle is at low altitudes and, for altitudes above 160 km, the brightness decreases exponentially with increasing altitude and with a scale height of 35 ± 2 km; this is appropriate to atomic oxygen at a thermospheric temperature $\sim 600\text{K}$. Thus it has been deduced that the glow arises from an interaction between the spacecraft and the ambient atmosphere. The brightness varies with the size of the spacecraft; for the Shuttle it could be as much as ~ 100 kRayleighs.

The crucial property of the glow radiation is that it is strongest in the ram direction, that is in the direction of the spacecraft's velocity vector. Because the spacecraft is moving at a velocity of 8 km s^{-1} , it strikes ambient atmospheric oxygen atoms, providing each of them with an energy of $8 \times 10^{-19}\text{ J}$, or 5 eV. Not yet investigated for the Shuttle, but observed for other space vehicles, is a small enhancement of radiation in the wake direction, antiparallel to the velocity vector.

The brightness of the glow is enhanced after the operation of the Shuttle's thrusters, small rocket engines which control the vehicle's orientation. As determined from star occultation, the radiation is emitted in a layer 5-10 cm thick just beyond the spacecraft surface. The interpretation is that after catalysis on the surface of the spacecraft, excited (or metastable) molecules are ejected. The dimensions of the glowing layer are consistent with a radiative lifetime of the emitting molecule of about 5 ms. Thus the emitter could be nitrogen dioxide or, more likely, hydroxyl formed by $\text{O} (^3\text{P})^* + \text{H}_2\text{O}$ (adsorbed) $\rightarrow \text{OH} + \text{OH}$, the radiation being vibration-rotation bands in the Meinel system.

The results give cause for alarm. Any earth-viewing remote-sensing experiment, or any astronomical experiment, operating in the visible or IR aboard the Space Shuttle, may be subject to contamination from the Shuttle's glow. The effect places constraints on the viewing geometry of any low-light-level instrument carried aloft by the Space Shuttle. For example, on Spacelab 1, which is due to be launched on 30 September 1983, one experiment consists of an array of imaging spectrometers. Anticipating the effect, the location of this instrument on the payload was planned so that no part of the Shuttle surface was viewed. Operating from the ultraviolet to well into the IR, to 1.2 μm , this instrument is designed to study weak radiation from several atmospheric species. Although the main research programme will not be conducted when the instrument is viewing in the ram direction, a special study of the glow radiation will be conducted over the full wavelength range. Finally, this contaminating effect is worst at sunspot maximum, when the density of the Earth's upper atmosphere is greatest. □

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Fertilization *in vitro*

Chromosomal abnormalities in human embryos

from R. G. Edwards

CONVINCING and accurate data on the incidence of chromosomal abnormalities in human embryos growing *in vivo* or *in vitro* have been hard to gather. Difficulties have arisen in making clear and unequivocal chromosomal preparations from the few mitoses present in preimplantation embryos, and the incidence of abnormalities such as monosomy, trisomy, mosaicism and polyploidy during these early stages of growth has not been established. There is nevertheless little doubt that these forms of chromosomal imbalance could be very frequent after fertilization *in vivo* or *in vitro* in some circumstances, for example in embryos of older mothers.

The paper by Angell and collaborators in this week's *Nature* (p.336) reflects the difficulties found by others in establishing the correct karyotypes of cleaving human embryos. Most embryos could not be classified, but two notable and novel observations were made. Two of the eleven embryos observed were haploid or near-haploid, although one was classified only by DNA microfluorimetry. The other was undoubtedly gynogenetic or parthenogenetic in origin, since the three classifiable mitoses were maternal 22X-17. This remarkable embryo thus displayed two anomalies simultaneously, revealing a non-disjunctional event in meiosis or immediately post-fertilization and an ability of haploid embryos to reach the seven-cell stage. It provides concrete grounds to confirm predictions that early human development can begin without fertilization — a point for ethicists to ponder — and that monosomy might occur with some frequency in fertilized eggs.

The implications of these observations for clinics working on human fertilization *in vitro* are less certain. We found no haploid embryos among more than 25 examined chromosomally, even though exact counts could not be made in most of them. Eggs with one pronucleus are rare — very rare — during fertilization *in vitro*, especially in comparison with the incidence of haploidy reported by Angell *et al.* Their methods might encourage the pathogenetic activation of oocytes; for example, the use of nitrous oxide for the pneumoperitoneum during laparoscopy for oocyte recovery, and conditions in the culture medium as oocytes are carried to a nearby laboratory might contribute. Their unyielding treatment of patients with clomiphene, human chorionic gonadotropin and ultrasound 19 hours later could lead to unpredictable follicular responses and the collection of unripe oocytes.

Two other unbalanced embryos were

found — a 47XY + D and another with metaphases apparently containing 44, 45 and 46 chromosomes respectively. This evidence was not unequivocal. There was some chromosome scatter from other metaphases in both embryos, and the blastomeres were uneven in one of them. Most embryos were unfortunately classified by fluorimetry rather than karyotypes.

It would be premature to relate the ex-

tremely high incidence of chromosomally imbalanced embryos to other work on fertilization *in vitro* until more evidence is forthcoming. No one doubts that many such embryos conceived *in vivo* and *in vitro* fail to develop to full term or even to implantation, and occasional trisomic, monosomic and triploid abortions have been reported after fertilization *in vitro*. There is a wealth of knowledge to be gained on the exact incidence and type of chromosome disorders in early human embryos conceived in various circumstances, for example in different age-groups of patients and in various clinical conditions. □

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Marine technology

Boiling deep beneath the ocean

from Roger N. Anderson

It is ironic that the oldest marine geological technique has presented the most recent surprise from the mid-ocean ridges. High technology has been responsible for a long series of discoveries that has remoulded our ideas of how the sea floor cools at the volcanically active Mid-Ocean Ridge. Bottom television, deep-sea drilling and deeply towed remote-sensing packages have been the kinds of tool that first discovered, then described, the 'Black Smoker' hot springs which pour forth 350°C metal-laden water onto the sea floor beneath up to 2 miles of ocean. These hot springs, found active for the first time in 1979, give us a glimpse of how metal deposits, such as massive sulphides, are formed.

Now a technique used on HMS Challenger to collect the first rock samples from the deep sea floor of over 100 years ago has delivered new scientific wonders. A 'dredge' is merely a large bucket dragged blindly across the sea floor and connected to the ship on the surface only by wire rope. J.R. Delaney and B.A. Cosen (*Mar. Technol. Soc. J.* 16, 62; 1982) report inclusions, or little bubbles, of super-high-salinity water in basalts dredged from the Mid-Atlantic Ridge. They conclude that boiling has taken place in the hot spring system that generated the fluid inclusions. Boiling has never before been found beneath the deep sea for the weight of two miles of water was thought to make it impossible.

For boiling to occur on the Mid-Atlantic Ridge, hot spring water must move upwards from its heat source — magma within a volcano — faster than it loses heat to the rock during its traverse to the surface. This requires a chimney, such as those observed by a diving submersible on the East Pacific Rise crest (Rise Project Group, 1980). Any good chimneysweep will tell you that hot vapour mixing with

cold will precipitate a good coating on the wall of a chimney.

On the Mid-Ocean Ridge, this coating is made up of metals and, as Delaney and Cosen point out, boiling changes the kinds of metal deposited as the hot spring fluid billows forth. At some depth within a Mid-Atlantic Ridge chimney, boiling occurred in the past when the temperature of the hot water became high enough to intersect the liquid-vapour phase transition curve. (If, however, hydro thermal fluid passing up a chimney intersects the sea floor before reaching the *P-T* boiling curve, no steam will occur.) The steam that then formed left behind liquid of very high salinity, just as the Great Salt Lake formed by evaporation. Some of that liquid ended up as fluid bubbles in the rock dredged, fortuitously, millions of years later.

But, what is really exciting is what Delaney and Cosen predict the steam carried to shallower parts of the chimney — gold and silver! In mines on land, lead, copper and zinc are found in the portion of high-salinity chimneys deeper than the boiling and gold and silver are found in the steam-dominated shallow parts. But don't get your pan out yet! There is probably no mother lode in the deep sea floor that any one could reach even with futuristic mining equipment. But scientific tools such as those which might be lowered into a deep-sea drilling borehole or down a chimney by a diving submersible might find a steam vent where we could observe precious metal deposits forming *in situ* for the first time ever. Then we might learn of places on land to look — places we haven't thought to look before. □

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Leprosy

Awakening the unresponsive lymphocyte

from Barry R. Bloom

FROM ancient times leprosy has evoked singular images of horror and fascination. Many aspects of the disease remain mysterious to this day. For example, *Mycobacterium leprae* remains one of the very few pathogens of man that cannot be grown in culture. The mode of transmission and the nature of the long latency remain unknown. The reasons why leprosy disappeared from Europe at the end of the last century, yet is currently increasing in some developing countries, remain unclear, although the latter may be partially explained by the emergence of drug-resistant organisms.

There has been a renaissance of scientific interest in leprosy, undoubtedly one reason being that it offers extraordinary possibilities for gaining insight into immunoregulatory mechanisms in man. Leprosy is a spectral disease that presents a diversity of clinical manifestations¹. At one pole of the spectrum, tuberculoid leprosy, patients develop high levels of specific cell-mediated immunity, which ultimately kills and clears the bacilli in the tissues, although often with concomitant immunological damage to the nerves. At the lepromatous pole patients exhibit a selective unresponsiveness to antigens of *M. leprae*, and the organisms ineluctably multiply in the skin, often to extraordinary numbers — as many as 10^{10} g⁻¹. It remains unclear why the vast majority of people exposed to *M. leprae* develop no clinical disease, and why only a minority who develop clinical disease become lepromatous and remain immunologically unresponsive to the organism. Serological comparisons of the antigens of *M. leprae*

indicate that all known protein or glycoprotein molecules are either identical or cross-reactive with those of *M. tuberculosis*². Yet, many patients with lepromatous leprosy paradoxically show positive cell-mediated responses to antigens of the tubercle bacillus while remaining completely unresponsive to antigens of the leprosy bacillus.

One explanation for this selective unresponsiveness in lepromatous leprosy would be that one or a small number of unique antigenic determinants present on *M. leprae* may induce unresponsiveness by stimulating specific suppressor cells that inhibit the reactivity of lymphocyte clones capable of recognizing other specific or cross-reactive determinants. Despite the elegant elucidation of regulatory T-cell networks in model systems, the ultimate mechanism by which suppressor cells prevent competent lymphocytes from functioning remains unresolved. Experimental support *in vitro* for lepromin-induced suppressor activity from lepromatous patients has appeared; an adherent cell, presumably a monocyte, and a suppressor T cell identified by the suppressor-specific OKT8 antigen have been implicated in some circumstances^{3,4}. Consistent with this model is the finding that OKT8 T cells predominate in lepromatous lesions^{5,6}.

Recent findings in experimental models indicate that mitogen-, alloantigen- or hapten-induced suppressor cells inhibit proliferation of T lymphocytes by blocking the production of interleukin 2 (IL-2)⁷⁻⁹. IL-2 is a lymphokine that is produced by activated T cells and binds to receptors expressed on stimulated, but not resting, T

cells, inducing them to proliferate. Malkovsky *et al.*⁹ have obtained a soluble suppressor molecule from T cells of mice rendered unresponsive to chemical allergens which mediates suppression by blocking IL-2 production. This factor is neither antigen-specific nor restricted by the major histocompatibility complex.

In this issue of *Nature*, Haregewoin *et al.*¹⁰ provide evidence that lymphocytes from lepromatous leprosy patients cultured with *M. leprae* fail to produce IL-2. More important, addition of supernatants from mitogen-stimulated lymphocytes known to contain IL-2 restores, at least partially, the ability of lymphocytes from lepromatous patients to respond to *M. leprae* antigens. This indicates that some T cells reacting to *M. leprae* exist in the blood of lepromatous patients, an observation consistent with the findings that depletion of OKT8 suppressor cells restores *in vitro* lepromin responsiveness in a portion of these patients⁴. These results establish that the defect in lepromatous leprosy cannot be due to an absence of lepromin-responsive T cells. Further, this work represents the first report of some degree of restoration of immunological competence in a disease-associated state of immunological unresponsiveness by means of lymphokines.

In recent years several vaccines have been developed that can produce positive skin tests in previously immunologically unresponsive lepromatous leprosy patients. The first of these vaccines, developed by Convit and his colleagues¹¹ in Caracas, Venezuela, contains killed purified armadillo-grown *M. leprae* mixed with living BCG. The results of immunotherapeutic vaccination of several hundred lepromatous leprosy patients indicate that more than two-thirds become immunologically reactive and rapidly clear the acid-fast bacilli from the tissues. In India, Deo and Bapat¹² and Talwar have reported that two strains of cultivatable mycobacteria can overcome unresponsive-

100 years ago

The Arctic Meteorological Station on the Lena



Russian Meteorological Station at Sagastyr, at the mouth of the Lena, 73° 22' 30" N. lat., 144° 14' 46" E. long.

The house of the station is obviously very small; and when looking at Mr. Schütze's sketch of this small building, lost amidst the snow-plain, far from any communication with the civilized world, one cannot but admire the

devotion of those who have willingly submitted to remain in these inhospitable latitudes for scientific purposes.

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ness to *M. leprae* in lepromatous patients. The report by Haregewoin *et al.* would suggest that organisms which are cross-reactive with *M. leprae* but have unique determinants capable of triggering T cells to secrete IL-2 or other appropriate lymphokines might be able to initiate the expansion of T-lymphocyte clones capable of mediating cell-mediated immunity and activating macrophages to kill and degrade *M. leprae*. These results may provide an important insight into a general mechanism for overcoming immunological unresponsiveness or suppression, with ramifications far beyond leprosy.

Beyond the scientific results presented, a further aspect of this work is worthy of note. The research of Haregewoin *et al.* was sup-

ported by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases, and the senior author is a recipient of a training fellowship from WHO. The work represents a collaboration between laboratories in Addis Ababa, Ethiopia and Oslo, Norway. This kind of linkage should serve as a model for accelerating access by researchers in developing countries to conceptual and technical advances in basic sciences to enable them to contribute more effectively to the solution of problems of both fundamental and practical importance. □

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Neuropeptides

Complex proteins coordinate simple behaviour

from Charles F. Stevens

THE small family of neuropeptides that controls the egg-laying behaviour of the marine snail *Aplysia*^{1,2} provides a striking example of a general trend in biological sciences: over the past decade, biological systems have come to seem more complex and less economical than we had thought. Traditional notions of their elegance and parsimony have been challenged on several fronts. For example, new findings about the structure of the genome — which has become incredibly tacky by old standards — and the daunting array of ionic channels responsible for the electrical activity of the nervous system, seem to demand new organizational principles. The synthesis and function of neuropeptides, as illustrated by the recent work of Richard Scheller, James Jackson and their collaborators at Columbia University, offers another such challenge; but in this case the organizational principle is not far to seek.

Egg-laying behaviour in *Aplysia* is initiated by a group of associated neurones called the bag cells. When these cells become active, the animal extrudes a string of eggs which it then arranges in a mass and attaches to the substrate with its mouth. The entire sequence of behaviour is known to be controlled by a related group of polypeptides, one of which is the egg-laying hormone (ELH).

ELH is the principal protein product of the bag cells, and the Columbia group began their analysis of the neuropeptides controlling egg-laying behaviour by cloning cDNA that codes for ELH. They were then in a position to use this cDNA as a probe to identify other related genes.

By this strategy, they uncovered a family of at least nine closely related genes involved in the control of egg laying.

Although the work of the Columbia group has many interesting facets, two im-

portant generalizations stand out. First, the neuropeptides are synthesized as part of a larger polypeptide and produced from it by proteolytic processing. Second, the overall behaviour is governed by products of a gene family, of which different members are expressed by different cell types. Thus, components of the egg-laying system that are functionally related clearly have a common evolutionary origin, revealed in the strong homologies between genes that define the family.

Scheller *et al.*² have now sequenced three representatives of this family. The genes code for products that share properties with polypeptides from other systems³: the polypeptide has a signal sequence characteristic of secreted proteins, and contains multiple functional peptides delimited by amino acid sequences recognized by trypsin-like proteases. Thus, on proteolysis, the polypeptide can give rise to multiple neuropeptides. The ELH gene expressed by bag cells contains sequences for ELH itself as well as three or four other small peptides thought to be involved in controlling the snail's behaviour.

The ELH polypeptide contains ten potential cleavage sites (pairs of basic amino acid residues) which, if they were used in all possible combinations, could produce 1,024 distinct polypeptides. Presumably most of these potential products are not made, but this calculation vividly illustrates how polypeptides such as the ELH precursor may produce a diversity of peptides from a single gene. These multiple products from a single gene may function, at least in part, to coordinate components of a complex system. ELH itself will mediate some, but not all aspects of egg-laying behaviour, and additional factors encoded by the ELH gene are necessary for the entire set of behaviours.

Two other representatives of this gene family are responsible for initiating egg-laying behaviour. They code for products known as the A and B peptides, and are about 90 per cent homologous with the ELH gene. The A and B peptides are expressed by cells in the atrial gland, a secretory organ associated with the reproductive tract, and initiate egg-laying behaviour by causing bag cell discharge. The discharge is produced in an interesting way. Binding of the appropriate ligand to bag cell receptors activates an adenylate cyclase which, through the production of cyclic AMP, ultimately causes bag cell channel properties to be modified so that a sustained discharge results. The bag cell discharge induces the release of ELH and its related peptides, and these neuropeptides in turn elicit the appropriate further changes in the nervous system that give rise to egg-laying behaviour. The evolutionary link between the ELH gene and the A and B peptide genes thus has a functional parallel in that different cells expressing the various members of the gene family all serve the final goal of eliciting a single complex behaviour pattern.

The Columbia group is now in a position to follow three important lines of research on the control of behaviour by neuropeptides. Cells expressing genes belonging to this family can be found by hybridization *in situ*. This method exploits homologies between members of a family by using a labelled cDNA of one member as a probe to detect mRNA copied from related genes in other cells. Insofar as evolutionary relationships between genes imply functional relationships between their products, the method can be used to uncover the network of neurones that subserve components of the egg-laying behaviour. Second, because expression of genes in this family must be developmentally regulated, the cDNA probes can be used to discover the sequence in which these genes are expressed and the relationships between them. Finally, cells such as the bag cells, expressing members of this gene family, can be used to investigate the processing of polypeptide precursors that result in the diversity of products and the coordinated release of multiple products.

Neuropeptide control of egg-laying behaviour involves a rather large variety of peptides and is, according to older concepts, rather complex. The molecular biological approach to this problem, however, seems to be revealing an underlying order and may explain why small peptides should be manufactured as parts of a polypeptide. Perhaps we are starting to perceive a new simplicity in the relationship between brain function and behaviour. □

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Meteorites

The demise of established dogmas on the formation of the Solar System

from P.K. Swart

FOR at least the past 10 years, a mild controversy has simmered in meteoritical and astrophysical circles regarding the strange distribution of xenon isotopes found in certain meteorites, known variously as CCF-Xe (carbonaceous chondrite fission), DME-Xe (demineralized acid etched) or Xe-X. Was this component formed in a supernova? Or was it merely a fortuitous combination of mass fractionation and other well established xenon reservoirs?

CCF-Xe is characterized by an excess of the light and heavy isotopes relative to ^{132}Xe and is contained within a mainly carbonaceous host phase constituting a small proportion of the total carbon in primitive meteorites such as Allende (C3V) and Murchison (CM2). The carbon isotopic composition of the carrier phase of CCF-Xe has provided us with no indication of any exotic origin for CCF-Xe, being essentially similar to Solar System values around ($\delta = -35$ per mill PDB). Now, the results of two crucial experiments, reported at a recent meeting*, have led the principal defendants in the argument over the origin of CCF-Xe to concur in favouring the supernova hypothesis.

The first of these results, reported by Lewis, Anders, Shinamura and Lugmair, involved the examination of the isotopic composition of elements, such as barium and samarium, comparable in mass to xenon. These elements should, according to the fission hypothesis, show CCF-type anomalies. No such patterns were found. The absence of such anomalies is strong evidence that CCF-Xe does not arise from fission, although one escape clause remains. During the harsh acid treatment to which these meteorites were subjected in order to concentrate the anomalous gases, some CCF-Xe may have been left behind, and the hypothetical anomalous Ba and Sm removed.

The second result was the discovery by Wright, Norris and Pillinger, in conjunction with the Chicago group, of nitrogen with an extreme depletion in the heavier isotope of nitrogen ($\delta^{15}\text{N} = -330$ per mill AIR). Such an isotopic composition is extremely unlikely to result through normal kinetic fractionation and although ion-molecule reactions are a possibility, most people think the nitrogen associated with CCF-Xe is the result of nuclear effects. If this is so, there now seems to be independent evidence that so-called CCF-Xe is derived from a supernova involving both p- and r-processes, as first suggested by Manuel *et al.* (*Nature* **240**, 99; 1972). The workers in this field must now come to

some kind of consensus on what to call this component.

While searching for a barium isotope anomaly, Lugmair uncovered excesses in ^{143}Nd and ^{142}Nd which he and his co-workers concluded were a result of the α -decay from ^{147}Sm and ^{146}Sm . Thus ^{146}Sm appears to have been 'alive and kicking' during formation of the Solar System.

A second interesting development relating to Xe was the discovery by Swart, Grady and Pillinger in conjunction with Anders and Lewis, that carbon associated with s-Xe (xenon formed principally by slow neutron capture) has an isotopic composition of +1,100 per mill (PDB): such a value clearly represents a remnant of a distinctive nuclear process. The value extends the range of δ values previously measured on all types of terrestrial and extraterrestrial material in the laboratory by almost two orders of magnitude, thus bringing respectability to the measurements of astronomers who claim to have been measuring such high ^{13}C values in extra-Solar System objects for some considerable time. The association of isotopically heavy carbon with s-Xe places interesting constraints on the formation of grains and the implantation of isotopic anomalous gases into them and I hope will spawn many attempts at interpretation. Nitrogen associated with this material appears from present measurements to be isotopically 'normal'.

Opportunity was provided for meteoriticists to hear and react to the controversial results of Thiemens and Heidenreich (*Science* **219**, 1073; 1983), which threaten established interpretation of the three-isotope oxygen system. Through experiments conducted in the laboratory (oxygen was dissociated to produce ozone), Thiemens was able to produce oxygen with an isotopic composition which did not fall on the line of slope 0.52 in the three-isotope plot of $\delta^{18}\text{O}$ against $\delta^{17}\text{O}$. Instead the data plotted on a line of slope 1; the effect is believed to be caused by optical shielding of the minor isotopes.

Hitherto data plotting on such a line have been interpreted by meteoriticists as being the result of mixing between a normal Solar System component and some ^{16}O -rich end member. Thiemens suggested that the experimentally produced fractionation along a line of slope 1 may indeed be an explanation for the same trend seen in meteorites. The fact that no nuclear effects are required could then very well explain the paucity of nuclear effects in the same FUN (fractionation and unknown nuclear effect) inclusions seen in other multi-isotope systems such as silicon (as reported

by Molino-Velsko, Mayeda and Clayton), titanium and magnesium (Hutcheon, Steele, Wachel, Macdougall and Phinney).

Surprisingly, most debate at the conference was directed not at the remarkable fact that effects which would be normally construed as nuclear could be produced in the laboratory, but at the mechanism for producing the ultraviolet for dissociating the oxygen-containing molecules. Thiemens suggested that T-Tauri stars, which are enhanced in UV by 10^4 , could be a source, a possibility later confirmed by Cameron during the discussion. This point was seized upon by several questioners. It is rather ironic that the major criticism levelled at workers in the field of carbon, hydrogen and nitrogen isotopes was that nuclear effects could not be ascertained because these elements only have two isotopes. With oxygen on the other hand, nuclear effects were clearly evident. Now the situation has apparently been reversed. Extreme enrichments and depletions in nitrogen and carbon have been classified as nuclear in origin, whereas oxygen shows only modest deviations from normal terrestrial values and data falling off the much heralded three-isotope plot can now be explained through simpler processes, not requiring a nuclear explanation.

An important point emerging from the various groups working on the stable isotopic composition of light elements (carbon, nitrogen and hydrogen) and their associations with noble gases in meteorites is that there are so far no simple and immediately obvious associations between distinctive carbonaceous phases, nitrogen and noble gases such as CCF-Xe. The distinctive isotopic composition of these light elements can be produced through a wide range of mechanisms whereas CCF-Xe may be limited to one scenario. Thus the discovery of light nitrogen in the iron meteorites reported by Prombo and Clayton is not necessarily the same nitrogen as that associated with CCF-Xe. For the same argument the apparent wide distribution of meteorites showing esoteric compositions of carbon and nitrogen reported at this meeting does not mean *a priori* associations with noble gas types. □

*The 14th Lunar and Planetary Science Conference was held in Houston on 14-18 March 1983.

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IRAS, the Infrared Astronomical Satellite

The Infrared Astronomical Satellite, launched earlier this year, is providing the first map of the entire IR sky. This article describes the operation and preliminary results of the IRAS mission.

THE launch of the Infrared Astronomical Satellite (IRAS) on 26 January 1983 marked the beginning of a new era for IR astronomy. The satellite is working well and has completed nearly one-third of an all-sky survey at four different IR wavelengths between 8 and 120 μm at a resolution of about 1×4 arc min. Following a week of in orbit engineering tests the telescope's protective cover was ejected on 1 February 1983. Since then an average of 1,000 galactic and extragalactic objects have been detected each day. Data from the telescope are being used to construct maps of the IR sky and a catalogue to include as many as 200,000 individual sources. A low resolution spectrometer records spectra from 8 to 23 μm of the brighter sources detected by the array. In addition to surveying the sky, IRAS can be used to make maps of small regions at both higher sensitivity and higher angular resolution.

IR observations fill a portion of the electromagnetic spectrum which until recently has been largely inaccessible. Their importance stems from the ability of IR radiation to penetrate the dust in the interstellar medium and because a large fraction of the total luminosity of many types of astronomical objects is emitted in the IR. This applies in particular to the study of star formation which takes place in dusty regions and, initially at least, at low temperatures. Because of these low temperatures most of the emitted radiation is confined to the IR.

Largely because of technological difficulties, unbiased all-sky surveys for faint far IR sources have never been performed before the IRAS survey except for two rocket surveys which, however, had little observing time available. Thus most of the contributions of IR observations to astronomy have come from limited observations of preselected objects.

Ground-based IR telescopes are limited in their ability to survey large areas of the sky, because most IR radiation is absorbed by the Earth's atmosphere and because of thermal emission noise from the atmosphere and the telescope itself. From its 900-km, near-polar orbit, however, IRAS views the sky without interference from the atmosphere below. Because the telescope is cooled to below 10 K it produces no significant internal thermal emission. The focal plane instrumentation is cooled to ~ 2.5 K to achieve maximum sensitivity. These low temperatures are maintained by a cryogenic system which, at launch, was filled with 72 kg of superfluid helium. This is the first time that superfluid helium has been used in a satellite experiment resulting in by far the lowest temperature of operation of any instrument in space.

Spacecraft and telescope instrumentation

The IRAS satellite consists of a spacecraft and a cryostat containing a cooled telescope with IR detectors mounted in its focal plane as shown in Fig. 1. Table 1 gives some of the relevant parameters characterizing the satellite. The spacecraft supplies electrical power, provides pointing control, and houses the telemetry and computer systems. The satellite's on-board computer is used to store and execute the instructions for each 12-h observing period. The resulting data are stored by one of two

on-board tape recorders. At the end of each observation period the recorded data are telemetered to the ground station at Chilton, UK and the instructions for the next observing period are sent up to the satellite. A small on-board read-only memory contains the instructions for emergency attitude control and other critical as well as routine functions.

The attitude control subsystem (ACS) points the telescope according to the observing plan, safeguards the telescope from excessive IR radiation from the Sun and Earth, keeps the solar panels pointed to the Sun and provides attitude data for position reconstruction for science data analysis. The control logic for the ACS is contained in the spacecraft on-board computer. Because gases from attitude control jets would produce unacceptable contamination of the cryogenic optical system, a non-propulsive reaction wheel pointing system is used. The combined torques from atmospheric drag and the venting helium gas lead to a gradual build up of angular momentum in the reaction wheels. This excess is transferred to the Earth's magnetic field by a set of momentum dumping coils. The spacecraft is powered by a solar panel array with nickel-cadmium batteries providing power for brief eclipse periods.

The telescope is an $f/9.6$ Ritchey-Chretien design with beryllium mirrors and a 57-cm aperture. A system of internal cold baffles reduces the system's response to off-axis radiation. External baffles and the relatively warm (100 K) sunshade reduce the heat load on the cold optical system from the Sun and Earth. The combined flux at the focal plane from the optical system and scattered radiation from the Sun and Earth are below the thermal emission of the zodiacal dust particles over a wide range of observing angles.

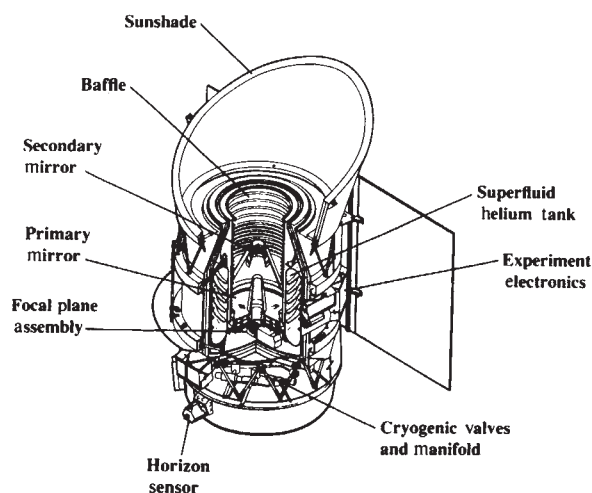


Fig. 1 A cutaway view of IRAS. The 57-cm aperture beryllium optical system is cooled to below 10 K by a superfluid helium cryostat and shielded from both solar and Earth emission by an elaborate set of baffles.

Table 1 Satellite characteristics

Mechanical	Overall length	3.6 m
	'Diameter'	2.2 m
	Mass at launch	1,076 kg
Orbit	Height	900 km
	Inclination	99°
	Period	103 min
	Precession	1° day ⁻¹
Cryogenic	Coolant	Superfluid helium
	Bath temperature	1,805 K
	Focal plane temperature	2.5 K
	Baffles temperature	10 K
	Sunshade temperature	100 K
	Outer shell temperature	195 K
	Helium consumption rate	2.4 mg s ⁻¹
	Helium at launch	71.6 kg
	End of cryogen	31 December 1983 ± 10 days
Optical	Telescope	2 mirror Ritchey–Chrétien
	Aperture	57 cm
	Focal length	5.5 m
	Plate scale	1.6 mm arc min ⁻¹
	Focal ratio	f/9.6
	Mirror material	Beryllium

The heat load on the cryogen of ~ 50 mW from the Earth and sky, the detector electronics, and from the spacecraft itself, will consume the cryogen after ~ 11 months of operation. When the cryogen is exhausted, the IR detectors will cease to function and the observational phase of the IRAS mission will be at an end.

Focal plane array IR experiments

The heart of the IRAS telescope is the focal plane assembly which contains the cold electronics. A schematic view of this assembly is shown in Fig. 2 and its characteristics are summarized in Table 2. The focal plane assembly contains a detector array for the survey, a low resolution spectrometer (LRS), and a chopped photometric channel (CPC).

The detectors in the survey array are arranged so that every source crossing the field of view is seen by at least two detectors in each wavelength band. As described below, this feature helps in discriminating between real signals and spurious noise pulses. High-energy particle hits from cosmic rays and exposure to Van Allen belt protons and electrons produce sharp spikes and substantially change the responsivity of the detectors for considerable periods of time following the end of an intense exposure. For this reason, the signals from the IR detectors are passed through on-board analogue circuits which detect and remove nuclear particle-induced glitches. This deglitching is followed by conventional low-pass filtering, before data storage. Although the deglitcher generally works well, there are some areas, such as in the South Atlantic Anomaly, where the nuclear radiation is too intense to be coped with. Major responsivity changes which accompany periods of such intense radiation are annealed by briefly applying high detector bias while small residual effects are corrected using frequent calibration data.

The LRS operates along with the survey array and is triggered to extract a source spectrum each time a sufficiently bright object passes over the survey array. Its wavelength and resolution characteristics are also summarized in Table 2.

The CPC uses an internal chopper to obtain absolute and differential photometry of selected objects. It simultaneously maps IR sources in two wavelength bands from 41 to 63 μm and from 84 to 114 μm . The CPC provides a higher angular resolution than the survey array and its internal choppers avoid the need for signal modulation by scanning. Its circular field of view with a diameter of 1.2 arc min is matched to the diffraction

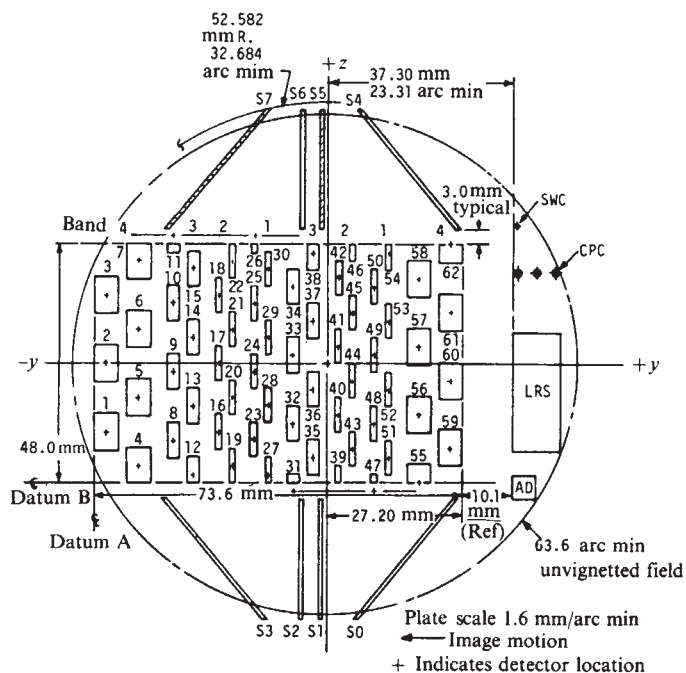


Fig. 2 A schematic view of the focal plane. There are 62 IR detectors in the main survey array covering the wavelength range from 8 to 120 μm in four colour bands. The LRS is basically an 'objective prism spectrograph' and is housed behind the large rectangular aperture. The CPC is used for making higher resolution maps of individual sources at 50 and 100 μm .

limit of the telescope at $\sim 80\ \mu\text{m}$. This makes the CPC well-suited for mapping areas of the sky with dimensions of a few arc minutes, with a clear tradeoff between sensitivity and map area.

The eight visible light detectors mounted near the sides of the focal plane assembly provide precise attitude information to the spacecraft computer for later use by the ground data processing computers. These data when used in conjunction with data from other spacecraft attitude sensors, enable the ground-based computers to reconstruct the boresight of the telescope to an absolute accuracy better than 20 arc s.

Survey

The major goal of IRAS is to make an all-sky IR survey, stressing reliability and completeness. The results will be published as a catalogue of point sources and graphical displays. The sensitivity limits, set arbitrarily at a signal-to-noise ratio of 10, are 0.7, 0.65, 0.85 and 3.0 Jy at 12, 25, 60 and 100 μm respectively. At this limit, 99.8% of the sources in the catalogue should be real, and no more than 2% of all real sources above that limit will have been missed except in limited regions of very high source density such as the galactic plane and the Magellanic Clouds.

The sensitivity limit is determined by detector responsivity and noise from the associated feedback resistors. In and near the galactic plane, the limit is determined by confusion from the large source density in the field of view and by increased diffuse background radiation. Reliability and completeness are being achieved by making repeated detections in each wavelength band. Confirmation occurs between sets of detectors on each passage over a source, then between detections made in consecutive orbits and finally between pairs of orbits separated by about a week. These repeated observations allow discrimination between distant and nearby objects such as dust particles, orbital debris, asteroids, and planets. Discrete sources are detected in the data stream of each single detector by an algorithm that recognizes point sources and small extended sources. Source confirmation takes place in each wavelength

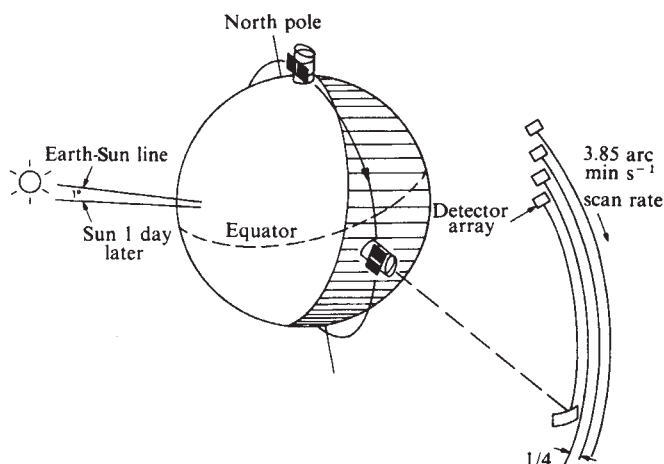


Fig. 3 A schematic drawing of the IRAS orbital geometry. The orbital altitude, 900 km, and inclination, 99°, combined with the Earth's equatorial bulge lead to an orbital precession of about 1° day^{-1} . As a result, the orbit normal always points towards the Sun as the satellite orbits above the Earth's terminator. By pointing the satellite radially away from the Earth, the cold telescope is more easily shielded from the heat loads from the Sun and Earth while providing natural scanning motion across the entire sky in about six months.

band separately. Merging of observations in different colour bands takes place after the existence of a given source has been established in at least one band. A map of diffuse extended emission with 2×2 arc min resolution will also be produced as a separate product.

The time needed to complete the survey is determined by the fundamental design of IRAS and by its orbit (see Fig. 3). IRAS scans long arcs across the sky at approximately 90° from the direction to the Sun. Due to precession of the orbit this great circle rotates over the sky at the rate of about 1° day^{-1} , which makes it possible to cover the entire sky in about 6 months.

Among data products that will be produced in addition to the four-colour survey catalogue of point sources are: (1) a catalogue of spectra of the stronger, isolated sources obtained with the LRS. (2) Large scale total flux maps of the sky to be reconstructed from the co-added detector output. (3) A catalogue of small extended sources (<8 arc min) detected in the survey. (4) Subsets of the primary catalogue selected on specific criteria such as all IRAS sources associated with catalogued galaxies. Extended source fields selected for particular attention include the galactic plane to a latitude of $\pm 10^\circ$, and smaller fields including the Andromeda Galaxy, the two Magellanic Clouds, M33, and the Orion nebula region.

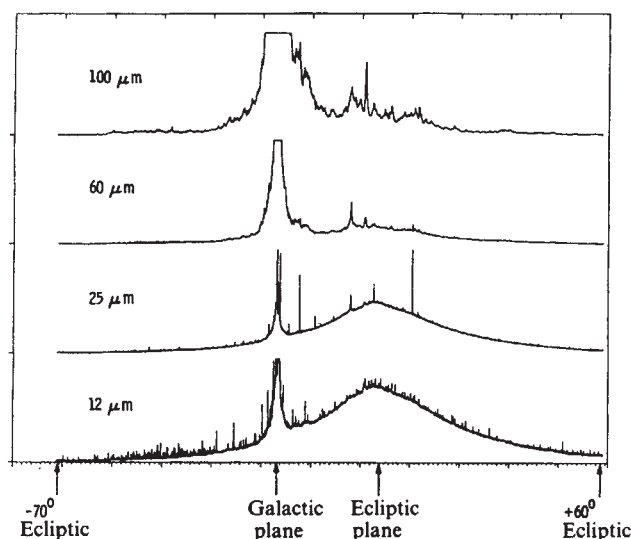


Fig. 4 An analogue reconstruction of detector outputs for a typical IRAS survey scan. The scan is made at constant angles with respect to the Sun-spacecraft line and thus cross-perpendicular to the ecliptic plane. The 12 and $25 \mu\text{m}$ tracings show very clearly the strong, relatively warm thermal emission from the zodiacal dust in the ecliptic plane. This emission decreases in importance at the longer wavelengths relative to the cooler emission from dust in the plane of the galaxy and to emission from molecular cloud regions near that plane. The small features in the long-wavelength tracings are reproducible and represent real features of emission from the sky (excepting the levelled peaks, where the data went off the scale of the plotter). The increased high-frequency noise in the lower tracing just to the left of the crossing of the galactic plane is caused by the effects of the particles in the north polar horns of the Van Allen radiation belts.

Pointed observations

In addition to the survey, a programme of pointed observations using the survey array and the CPC has been developed by the IRAS science team. The purposes of these observations are: (1) to gain sensitivity by increasing the integration time through co-addition of repeated scans and by pointing the most sensitive detectors at the specified object and; (2) to observe sources at a higher angular resolution than achieved during the survey. Sensitivities an order of magnitude better than that in the survey can be achieved by observing in this manner. To this date, some 2,000 pointed observations of selected objects have been done.

Currently planned programmes generally follow the theme of the study of star formation in a variety of conditions and circumstances. They also include photometric studies of well-known active or peculiar galactic and extragalactic sources.

Table 2 Characteristics of the focal plane assembly

Centre wavelength (μm)	No. of detectors	Detector field of view (arc min $\times$ arc min)	Wavelength interval (μm)	Detector material	Dwell time (ms)	Average 10σ sensitivity (Jy)
Survey array						
12	16	0.75×4.5	8.5–15	Si:As	190	0.7
25	15	0.75×4.6	19–30	Si:Sb	190	0.65
60	16	1.5×4.7	40–80	Ge:Ga	390	0.85
100	15	3.0×5.0	83–120	Ge:Ga	780	3.0
Chopped photometric channel						
Band 1	1	1.2 (diameter)	84–114	Ge:Ga	1,000	7.0
Band 2	1	1.2 (diameter)	41–63	Ge:Ga	1,000	7.0
Low resolution spectrometer						
Band 1	3	5.0 (width)	8–13	Si:Ga	Resolving power 14–35	
Band 2	2	7.5 (width)	11–23	Si:As	14–35	

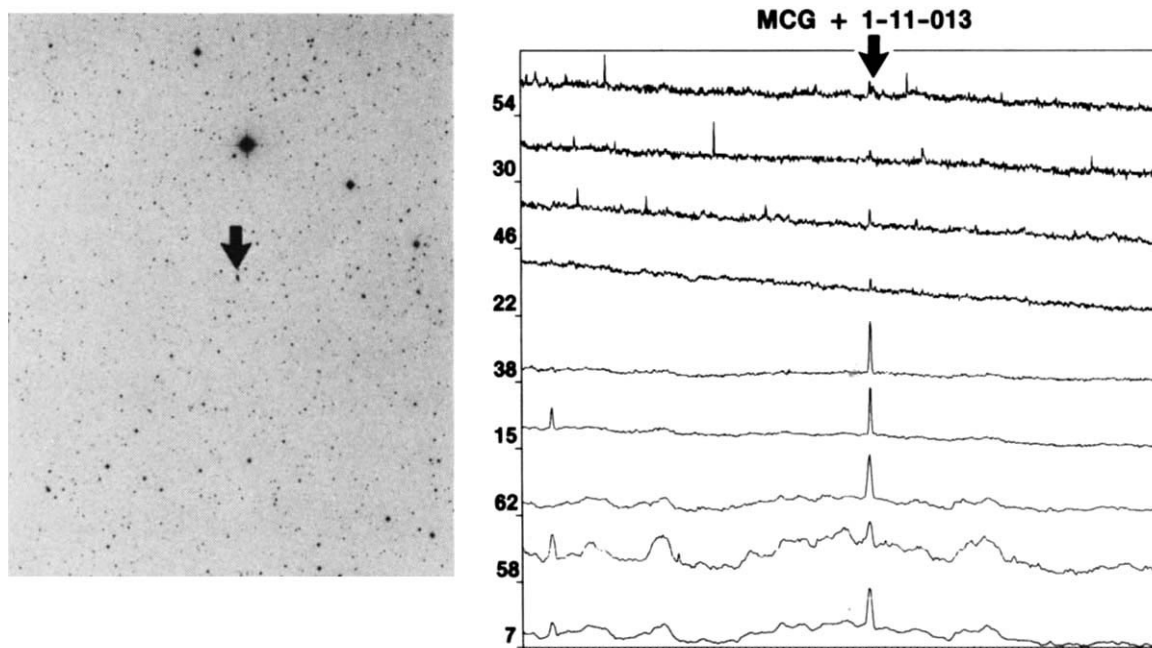


Fig. 5 The visual field (left) and IR signatures of the 15th magnitude galaxy MCG + 1-11-013 as the IRAS IR detectors passed over this portion of sky. The field of the photograph is about 1° . The IR signals are plotted against time, and show from top to bottom the signals at 12, 25, 60 and $100\ \mu\text{m}$ as the galaxy crossed the individual IR detectors. The time axis for each detector has been shifted so that a true celestial source appears at the same horizontal position in each detector stream. From the photographic magnitude and the measured IR flux, the ratio of luminosity at $60\ \mu\text{m}$ to that in the visible is ~ 10 . This ratio of IR-to-visible luminosities places this galaxy in the extreme high end of the known range of IR-to-visible luminosities in galaxies.

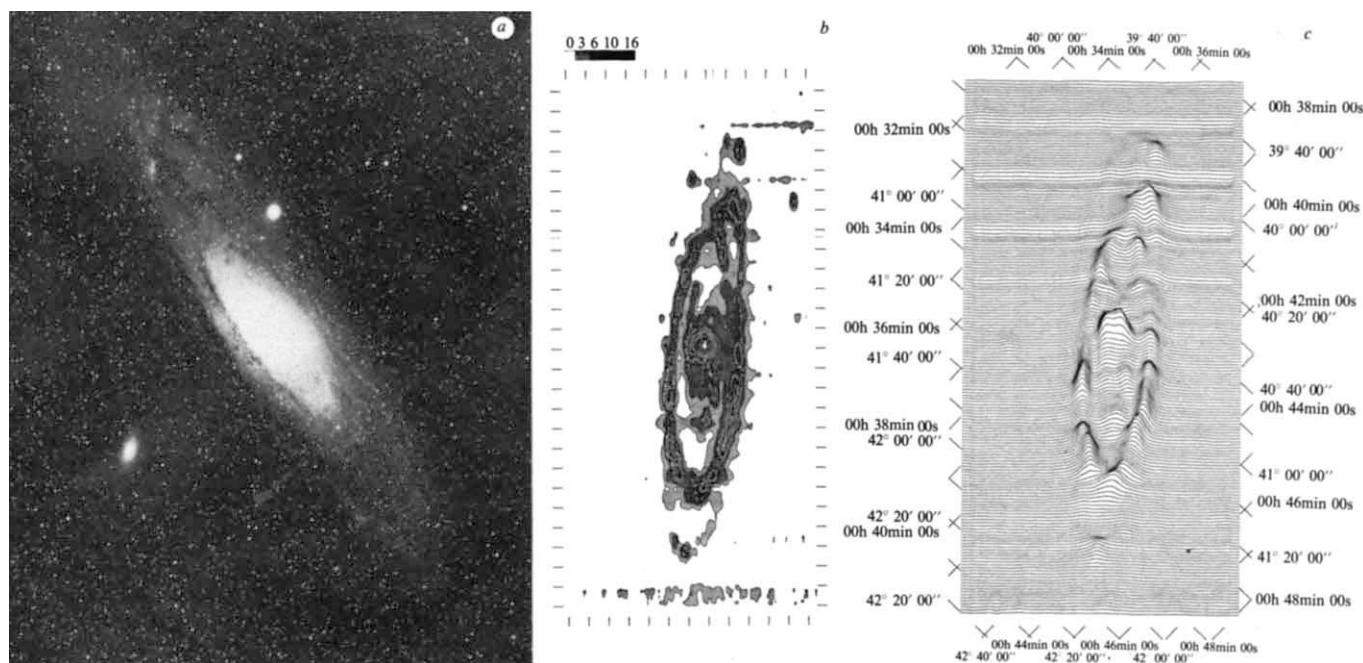


Fig. 6 A photograph of M31, the Andromeda galaxy, in the visual (a) and as it is seen in images generated from IRAS data at $60\ \mu\text{m}$ (b) and $100\ \mu\text{m}$ (c). Dominant structures in both IR images are the nucleus and outer ringlike structure. The dominant ring is at a radius of 9.5 kpc and is consistent with the ring of H II regions and H I seen in other studies. The outer area at a radius of 14 kpc along the major axis also coincides with an area of H I and H II regions. The nucleus is bright at $60\ \mu\text{m}$, $100\ \mu\text{m}$ and radio continuum but not in the 21-cm H I line. The total far IR luminosity of M31 is roughly 1% of the optical luminosity. The IR and visual luminosities of our Galaxy are about equal.

Maps of selected molecular clouds and of areas found in the survey will be used to study the formation of stars in various environments and search for low-luminosity stars in nearby clouds. Several interesting fields, such as areas of the Virgo clusters of galaxies, and potentially 'blank' fields have been selected for study at the highest sensitivities achievable. Finally, many Solar System objects, such as the comet Halley, will be specifically targeted for observations; of great interest in the

past month has been the discovery of a new comet by IRAS, named IRAS-Araki-Alcock.

Operations

The operations and control centre (OCC) at the Rutherford Appleton Laboratory (RAL) provides ground operations software for three main tasks: preparing the satellite's observing programme, communicating with the satellite during telemetry

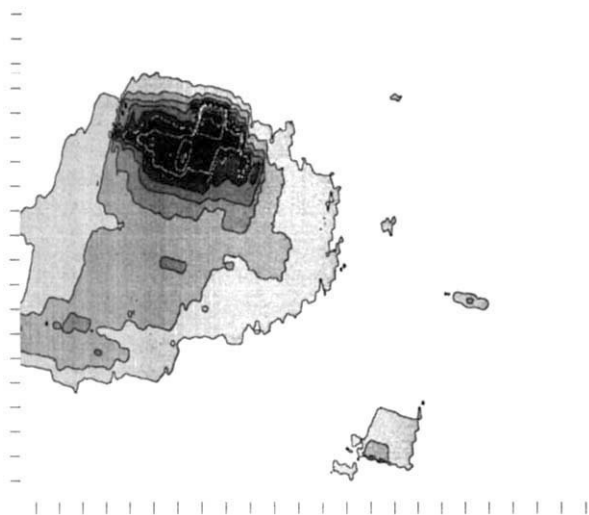


Fig. 7 A grey scale representation of the $60\text{ }\mu\text{m}$ IRAS observations of W1, a giant H II region in the northern Milky Way. W1 is a radio source first identified in the early radio continuum survey by Westerhout and is associated with the optically detected H II region Sharpless 171, at a kinematic distance of 1 kpc. The area shown is $\sim 2^\circ$ square.

passes, and processing the engineering data from the satellite. The first of these functions is especially time-critical in that data received during a given pass must be analysed quickly so that any necessary corrective action or recovery scans can be scheduled on the next prime pass 12 h later. A preliminary analysis facility at the site provides the means to carry out a quick-look evaluation of the scientific instrument performance, to assess the progress of the survey and to schedule pointed observations. Reduction of the CPC observations is also done at RAL.

The production of the final IRAS catalogue and its appendices will be done at the Jet Propulsion Laboratory using the IRAS Scientific Data Analysis System (SDAS). The IRAS data are transmitted to SDAS via a communications satellite and arrive there 6 h after ground receipt and pre-processing at the OCC. After the data have been calibrated and auxiliary functions such as satellite pointing reconstruction have been performed, the IR detector survey data are split into two streams. The first of these is searched for point and small extended sources (those sources that are roughly less than 8 arc min).

The second data stream for the survey data is used to build total intensity images of the diffuse IR emission associated with the zodiacal background, the galactic background, and other extended sources.

Preliminary results

At the time of writing, the survey is progressing according to plan. About 60% of telescope time is spent in survey work and the rest in pointed observations. A very small fraction of the data collected has been analysed beyond a quick-look.

Processing of IRAS data started shortly after the telescope cover was ejected. It is clear that the survey data are showing large numbers of objects both in and near the galactic plane and near the galactic poles. Portions of a survey scan are shown in Fig. 4. Because of the compressed scale, only large-scale features can be seen in these plots. However, emission from the galactic and ecliptic planes is evident. From those cases where scans were repeated over the same area of the sky, it is evident that the scans repeat in detail and that the structure seen in Fig. 4 is real.

Most of the time at SDAS since the cover deployment has been spent optimizing the survey processing system by repeated analysis of a small quantity of data known as the mini-survey. It is expected that full-scale processing of the survey data will begin in May.

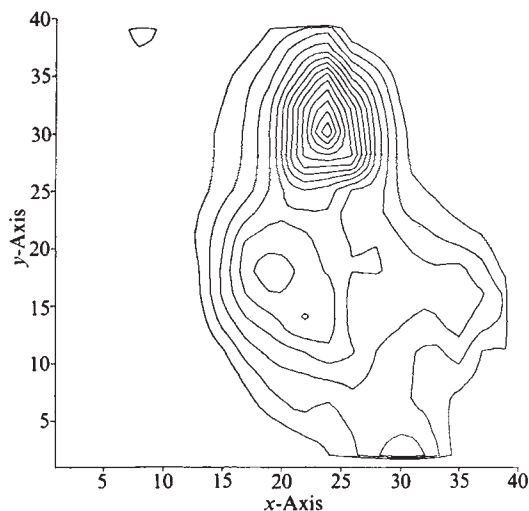


Fig. 8 A contour map (9 arc min square) of the radiation at $100\text{ }\mu\text{m}$ from the compact H II region G29.9-0.0 in the W43 complex obtained with the CPC.

The mini-survey consists of 12 separate coverages of an area of several hundred square degrees. Although this area is too small to permit careful statistical conclusions, the trends are clear. Between 15 and 20 galaxies have been observed which meet all of the tests needed for inclusion in the final catalogue. On this basis, one would predict that the final catalogue will contain nearly 10,000 galaxies. An example of a survey detection of a galaxy is shown in Fig. 5.

The survey array has been used in the pointed observations mode to map numerous galactic and extragalactic objects. Here we show only representative examples to indicate both the sensitivity and resolution of the system. Figure 6 shows a map of the Andromeda galaxy, M31. The total observing time required to obtain the data was 36 min. The most striking feature of the map, the strong ring of emission, is coincident with the ring of bright H II regions seen on optical photographs and with strong emission of atomic hydrogen seen in radio maps. Figure 7 shows a survey array map of the H II region molecular cloud complex W1. Previous IR studies of this object have concentrated on small point-like sources and largely ignored the more diffuse emission. The CPC has also produced numerous maps of galactic and extragalactic objects. Figure 8 presents a $100\text{-}\mu\text{m}$ map of the H II region G29.9-0.0.

The final catalogue will be published in the second half of 1984. However, some early results will be announced approximately twice monthly in *Nature* and in *Astronomy and Astrophysics* during the observing phase of the mission. In addition, a series of preliminary papers is being prepared for publication in the autumn of this year, with more detailed papers to appear as appropriate.

The United States, the Netherlands, and the United Kingdom are partners in the IRAS project. The US provided the cryostat, the IR telescope and the survey array of detectors, launched the satellite aboard a Delta rocket from NASA's Western Test Range at the Vandenberg Air Force Base in California and designed and operates SDAS. The Dutch provided the spacecraft and integrated it with the telescope, the uplink, downlink and on-board software, and the LRS and the CPC and their associated data reduction software. The UK conducts satellite command and data acquisition and provides quick look facilities from the ground station at the Rutherford Appleton Laboratory.

Although this paper was written by a small subset of scientists and engineers working on IRAS, a project the size and complexity of IRAS can only be successful through the work and coordinated efforts of a very large number of individuals who remain anonymous.

Evidence for a distorted solar core rotating with a 12.4-day period

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The periodicity recently discovered in the solar Doppler velocity may be due to an Eddington–Sweet current driven in the photosphere by a distorted gravitational potential rotating rigidly with a synodic period of 12.81 ± 0.10 days (12.4 days sidereal).

RECENTLY the solar velocity (averaged over the whole solar disk and measured spectroscopically as a Doppler shift) was found by the Birmingham group to vary sinusoidally with a 13.1 ± 0.2 -day period and with a $\sim 6.5 \text{ m s}^{-1}$ amplitude¹. This period is nearly the same as that originally found in the residuals of the 1966 'solar oblateness' observations². It was previously found³ that a good account of these old observations could be obtained from a model in which the distortion of the Sun was complex, requiring 2nd and 4th degree spherical harmonics ($l = 2, 4$) of 0, 1, 2 and 3rd order ($m = 0, \pm 1, \pm 2$ and ± 3) and this complex figure rotated rigidly over the solar surface, as a wave, with a synodic period of 12.81 ± 0.01 days (ref. 4).

The Birmingham observations were made over an 88-day interval simultaneously at two sites: Izana and Haleakala. The amplitude of the variation in velocity ($\sim 6.5 \text{ m s}^{-1}$) is 3 orders of magnitude too great to be accounted for as a simple radial motion of the solar surface. Transverse flow in the photosphere seems to be required.

The arguments developed in this article run as follows: a non-spherical solar figure seems to require a distorted gravitational–centrifugal potential and consequently a gravitational acceleration which varies over the solar surface. For mechanical equilibrium of the photosphere the radial gradients of pressure, density and temperature would vary over the surface. But these variations are incompatible with the requirements for steady radiation transport through the photosphere. The latter requirements dominate and the fluid is forced into a non-equilibrium state with accelerated transverse currents in the surface layers. These currents appear to be compatible with the Birmingham observations.

There are two key assumptions: first, that the 1966 data imply a rigidly rotating solar distortion and second, that the distortion implies a corresponding distorted gravitational/centrifugal potential.

Frequency intercomparison

Before discussing the circulation current (Eddington–Sweet) in the photosphere as a possible source of the anomalous periodicity in the whole-disk Doppler velocity, it is necessary first to establish the conformity of the period of this Doppler velocity with that of the rigidly rotating solar distortion. In the same context the 12.0715 ± 0.002 -day period found in daily sunspot numbers by Knight *et al.*⁵ seems to differ too much from the other two periods to be associated with them.

The period of the rotating distortion, 12.81 days, agrees nicely with the 13.1 ± 0.2 -day period in the velocity curve¹, but there seems to be a question about the validity of this agreement. Figure 2 shows that both the Izana and Haleakala data contain some very wild points, 'outliers', particularly in the Haleakala data¹. Note that all data are not of equal significance and the curve fitted (with a period of 13.15 days) is based on the Izana data only and it excludes data with day numbers less than 29.

A few wild points, interlopers not representing data, can severely pull the regression coefficients of a least square fit. Some form of robust fit is frequently used to eliminate outliers. The simplified assumption will be made here that the population of data points shown in ref. 1 consists of two classes of points: (1) a major class with normally distributed errors; and (2) a minor class (containing only a few members) with large residuals and containing no useful information. A robust method of iterative weighted fits is used with the weight zero applied to points having residuals greater than some arbitrary threshold. If the assumptions concerning the error distributions in the two data classes are correct, the fit should be stable under a decrease of the threshold, as some 'good' data are lost after the 'bad' data have been eliminated. This test is satisfied by the Birmingham data (see the first six lines of Table 1). The data were mechanically read from an enlarged copy of Fig. 2 of ref. 1.

The results of various robust least square fits are given in Table 1. These are four parameter fits and include the (linearized) frequency variation as one of the parameters. The results for Izana and Haleakala agree with each other in frequency, amplitude and phase. For an ordinary least-square fit (not robust) this agreement disappears with a phase difference of 19° and an amplitude ratio of 1.7.

The fit of the first line of Table 1 is plotted in Fig. 1 along with the data. Rejected points are dotted. Four of the rejected Haleakala points fall outside the diagram. From Table 1 this fit gives 13.29 ± 0.12 days for the synodic period, in excellent agreement with ref. 1. The computed errors in Table 1 are only formal, not corrected for data loss. They should be increased by ~ 15 – 20% for a 10 m s^{-1} residual upper bound.

Surface distortion

The 1966 observations² of the solar oblateness gave a P_2 distortion (Legendre polynomial) large enough to raise questions about the validity of Einstein's general relativity. But an enigmatic periodicity found later in the residuals could not be satisfactorily explained until 1976 (ref. 3) when it was found that a much better fit to the data could be obtained using the hypothesis that the surface was distorted in a complex fashion and that the distortion rotated rigidly over the surface (like a wave) with a synodic period of 12.81 ± 0.10 days (ref. 4).

These two fits to the 1966 data are shown in Fig. 2. The dotted curve assumes a simple solar oblateness (an $l = 2, m = 0$ or P_2 distortion). The data points are daily averages of the 'diagonal component' of the ellipticity corrected for atmospheric refraction, instrumental errors (mirror distortion) and faculae. (See ref. 4 for treatment of the data.) The sinusoidal variation with time of the dotted curve is due to the change in orientation (the angle P , ref. 2) of the solar axis relative to Earth's north.

The solid curve is obtained from the solution given in ref. 4, Table 1. The great complexity of this solid curve comes from the presence of the 2nd and 3rd harmonics of the basic

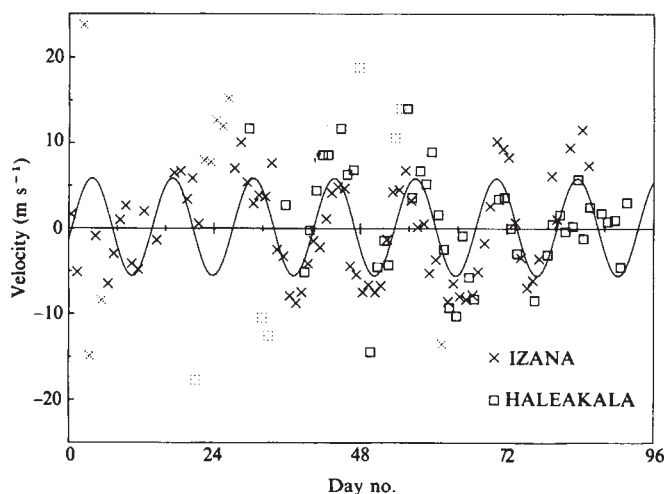


Fig. 1 Mean Doppler velocity of the solar surface. The data points, representing daily averages, are from ref. 1, Fig. 2. (Four Haleakala points fall off the chart.) The sinusoidal curve with a 13.3-day period is from line 1 of Table 1. The dotted points are data rejected by the computer under the robust procedure (see text).

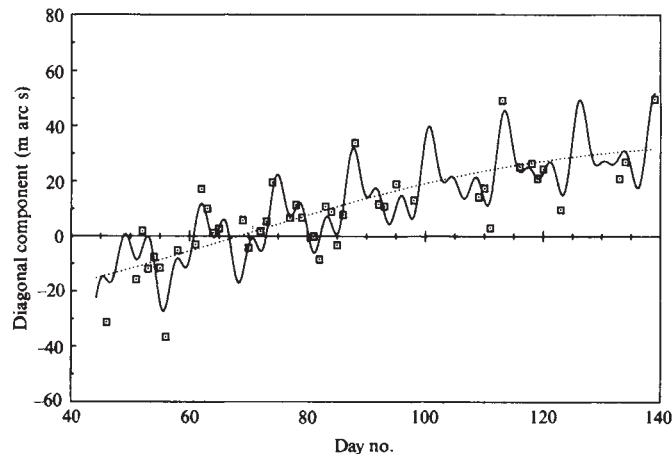


Fig. 2 The 1966 'oblateness data' fitted under the hypotheses show: first that the distortion is a simple oblateness (dotted curve) and second that the distortion is complex and is rotating rigidly and wave-like over the solar surface. The solid curve is from ref. 4, Table 1. The synodic rotational period is 12.8 days. The data points are daily averages of the 'diagonal component'. See ref. 4 for the treatment of the data.

frequency $(1/12.81) \text{ day}^{-1}$ and from the modulation of these periodic terms by the orientation angle P . The even and odd harmonic terms are multiplied respectively by $\sin 2P$ and $\cos 2P$.

The solar oblateness term ($m=0$) is present in both fits but the implications for Einstein's theory are different. To be significant with respect to the relativistic rotation of Mercury's perihelion the P_2 distortion (J_2 of the Sun if present in the gravitational potential) must be averaged over the period of the orbital observations (two centuries for the optical observations).

The purely P_2 distortion (dotted curve) seems to require a (permanent) rapidly rotating solar core. The solid curve seems to require a core distorted by a strong internal magnetic field and rotating with a sidereal period of 12.38 days (refs 6–8). In the latter case the solar core should nutate similar to a rigid body and it may also precess as a result of internal oscillations. Until these effects are understood the solar P_2 , and the implied J_2 , cannot be time-averaged. Thus there are no secure implications for the validity of general relativity. It is possible that these nutation and precession effects account for the disagreement between the 1966 results and those obtained in 1973 by Hill and Stebbins⁹. However, the two measurement techniques were very different and there may be some other explanation.

Circulation currents in the photosphere

From the early work of von Zeipel (1924) and of Eddington and Vogt it is known that static equilibrium with a distorted gravitational potential is generally incompatible with the requirements of radiative equilibrium inside a star¹⁰. The classically studied problem involves the deep interior and requires a rotating star. The centrifugal potential is the source of the distortion. Surfaces of constant potential are also surfaces of constant pressure and density. These pressure and density distributions (established on a short mechanical time scale) are incompatible with the requirements of energy balance, established on a long time scale. To avoid a pile-up or loss of energy in a small element of volume, without violating the requirements of mechanical equilibrium of the element, the fluid slowly circulates inside the star moving the volume element in question and keeping its temperature proper for mechanical balance.

In the Sun's photosphere the situation should be different. Despite the short mechanical time scale ($\sim 1 \text{ h}$) the time scale for establishing radiative equilibrium is even shorter (seconds). Hence conditions for radiative equilibrium are dominant and are incompatible with mechanical conditions for equilibrium. This should result in the acceleration of surface currents.

Table 1 Least square fit (robust) of $c + a \cos 2\pi\nu t + b \sin 2\pi\nu t$ to data

Data	Maximum residual* (m s^{-1})	N†	Frequency (μHz)	a (m s^{-1})	b (m s^{-1})	σ ‡
Combined§	10	115	0.871 ± 0.008	-5.6 ± 0.6	-0.9 ± 0.6	4.29
	8	110	0.873 ± 0.007	-5.4 ± 0.5	-1.5 ± 0.5	3.94
	6	93	0.870 ± 0.006	-5.9 ± 0.5	-1.4 ± 0.5	3.12
Combined	10	97	0.873 ± 0.011	-6.1 ± 0.6	-1.1 ± 0.7	4.15
	8	92	0.863 ± 0.010	-5.9 ± 0.5	-1.5 ± 0.6	3.72
	6	84	0.854 ± 0.009	-6.4 ± 0.5	-0.4 ± 0.6	3.17
Izana¶	10	71	0.874 ± 0.009	-5.6 ± 0.7	-0.7 ± 0.7	4.18
Haleakala #	10	44	0.874 ± 0.02	-5.4 ± 1.0	-1.2 ± 1.2	4.48
Princeton**	—	—	0.903 ± 0.007	—	—	—

* Threshold for the elimination of data. Maximum allowed residual of retained data.

† Number of data points retained.

‡ r.m.s. error of retained data from residuals (unbiased estimated error).

§ Combined data (Izana + Haleakala); total available points 128; $t=0$ for day number $d=50$.

|| Data with $d < 29$ rejected.

¶ Izana data only, including points with $d < 29$; 80 points total.

Haleakala data, including points with $d < 29$; 48 points total.

** From the Princeton 1966 'solar oblateness' measurement (synodic period of 12.81 days, improved analysis of 1981⁴); original value³ (1976) $\nu = 0.916 \pm 0.009 \mu\text{Hz}$ (synodic).

To simplify the calculations and the discussion, the effects of magnetic fields and of fluid motion in the photosphere are neglected for the unperturbed state. The first-order perturbation equation is

$$\rho \ddot{\xi} + \nabla p' + \rho' \nabla \phi + \rho \nabla \phi' = 0 \quad (1)$$

where ϕ , p and ρ are the unperturbed gravitational potential, pressure and density and the perturbations (Euler, that is, at fixed coordinates) are primed. Also ξ is the infinitesimal displacement vector of a fluid element. The unperturbed Sun is assumed to be spherically symmetric and ϕ' is assumed to be without a spherical part (no $l=0$ spherical harmonic). ϕ' is assumed to be given (determined by the rigidly rotating distorted core).

From the requirements of mass conservation, the density perturbation ρ' is given by

$$\rho' = -\nabla \cdot \rho \xi \quad (2)$$

Imagine the perturbation ϕ' switched on slowly enough to leave the Sun in a mechanical equilibrium state. The ξ can be neglected and equation (1) yields two equations:

$$p' + \rho \phi' = 0 \quad (3)$$

$$g \rho' - \left\{ \frac{d\rho}{dr} \right\} \phi' = 0 \quad (4)$$

obtained respectively by operating on equation (1) with rx and ∇x . Here $g = d\phi/dr$ is the unperturbed gravitational acceleration.

From the condition for static equilibrium of the unperturbed Sun, $dp/dr + g\rho = 0$, equations (3) and (4) can be written $p' = -\eta (dp/dr)$ and $\rho' = -\eta (d\rho/dr)$ where $\eta = -\phi'/g$ is the radial displacement of a surface of constant $\phi + \phi'$. This shows (von Zeipe) that the constant potential surfaces are also surfaces of constant pressure and density (and, for uniform composition, of constant temperature).

In the deep interior of the Sun the fluid displacements are adiabatic and

$$\delta p = c^2 \delta \rho \quad (5)$$

where $c^2 = \gamma p/\rho$ is the velocity of sound (squared) with $\gamma \approx 5/3$ the specific heat ratio. Here δp and $\delta \rho$ are lagrangian perturbations (moving with the fluid) and $\delta p = \rho' + \xi \cdot \nabla \rho$, and so on.

Combining equations (3), (4) and (5) gives

$$(\rho' + \xi \cdot \nabla \rho)[(dp/dr) - c^2 (d\rho/dr)] = 0 \quad (6)$$

Below the outer convective shell the second factor is non-zero and the first factor in equation (6) must vanish, $\nabla \cdot \xi = 0$ and $\delta \rho = 0$. The radial part of $\xi = \eta$, and matter moves along with (slowly moving) surfaces of constant $\phi + \phi'$ with neither a change in density nor of pressure.

In the convective zone the second factor in equation (6) is nearly zero. As a consequence, the buoyancy force on a displaced fluid element is substantially zero.

In the photosphere the buoyancy force on a small slowly moving fluid element is zero for a different reason. Such an element quickly comes to thermal equilibrium with its surroundings. From equation (6) this defines a modified sound velocity $\tilde{c}^2 = (dp/dr)/(d\rho/dr)$ and an 'effective γ ' (ref. 11), $\tilde{\gamma} = \tilde{c}^2 \rho/p$. We assume that throughout the convective zone and the photosphere the second factor in equation (6) is substantially zero. Substituting $\tilde{c}^2$ in equation (5) yields $\delta p = \tilde{c}^2 \delta \rho$ and

$$p' \approx \tilde{c}^2 \rho' \quad (7)$$

The mass displacement vector $\rho \xi$ is conveniently expressed in terms of three scalar fields, u , v and w , as

$$\rho \xi = \nabla u + Qv + Lw \quad (8)$$

respectively, the sum of a gradient, a poloidal displacement and a toroidal displacement. Here $L = r \times \nabla$ is the infinitesimal rotational operator and $Q = \nabla \times L$. The algebraic properties of these

operators have been tabulated elsewhere¹². Both L and Q generate divergenceless flows and L has no radial part.

From equations (2) and (7) we have

$$\rho' = -\nabla^2 u \quad (9)$$

$$p' = -\tilde{c}^2 \nabla^2 u \quad (10)$$

Operating on equation (1) with L yields $\ddot{w} = 0$ or $w = 0$ after including neglected damping effects. Thus there is no toroidal flow.

By operating on equation (1) with rx and ∇x we obtain respectively¹²

$$\ddot{u} - \frac{\partial}{\partial r} r \ddot{u} + p' + \rho \phi' = 0 \quad (11)$$

$$r \nabla^2 \ddot{u} + g \rho' - \frac{d\rho}{dr} \phi' = 0 \quad (12)$$

From equations (10) and (11)

$$\tilde{c}^2 \nabla^2 u - \ddot{u} = \rho \phi' - \frac{\partial}{\partial r} r \ddot{u} \quad (13)$$

This is the inhomogeneous wave equation for the propagation of sound. The right side of equation (13) represents the sound source. With sonic propagation times of ~ 1 h in comparison with the ~ 12 -day period of ϕ' the scalar field u smoothly follows the variation in ϕ' and $\ddot{u}$ in equation (11) can be neglected in comparison with p' .

If $\ddot{u} = 0$, we again obtain equations (3) and (4), the conditions for quasistatic-equilibrium, and the perturbed pressure, density and temperature are functions of the perturbed potential ($\phi + \phi'$). But for radiative equilibrium in the photosphere ρ and T must be functions of optical depth.

If both mechanical and radiative equilibrium were to hold simultaneously the radial displacement of the photosphere induced by ϕ' , namely $n = -\phi'/g$, would occur without a radial expansion or compression of the photosphere. But this would require $d\eta/dr \approx 0$ which is impossible.

From equations (9) and (10) only the gradient part of $\rho \xi$ perturbs ρ and p (Euler). A radial displacement of a thin outer shell (including the photosphere) occurs without a compression or expansion of the shell if (in the shell) $\nabla \cdot \xi_u = 0$, where $\xi_u = \nabla u/\rho$ is the gradient part of ξ and the radial part of ξ_u is constant. These conditions preserve the radiation balance in the shell, neglecting curvature effects. Neglecting the effect of the perturbation of g the convective energy transport is also left in balance. For a shell thickness of $\nabla r \approx 5 \times 10^7$ cm the heat capacity of the shell gives a thermal relaxation time of ~ 20 minutes.

This shell is adopted as the zone for which ξ_u is constant and $\nabla \cdot \xi_u = 0$. Below the shell, mechanical equilibrium holds. It holds also at the bottom of the shell, $r = r_1$, where $\xi_u + \phi'/g = 0$. Inside the shell the second and third terms in equation (12) can be written $-(dp/dr)g(\xi_u + \phi'/g)$. Assuming that ϕ' is a spherical harmonic of degree l , ϕ'/g can be expanded to first order about r_1 to give

$$-(l-1)(dp/dr)\xi_u g \frac{(r-r_1)}{r_1}$$

for the above.

Substituting this expression in equation (12) and neglecting the (small) term $-l(l+1)\ddot{u}/r^2$ in the laplacian gives

$$\frac{\partial}{\partial r^2} (r \ddot{u}) = (l-1) \left(\frac{d\rho}{dr} \right) \xi_u g (r-r_1)/r \quad (14)$$

and this can be integrated from r_1 to r to give $\partial/\partial r (r \ddot{u})$.

Equation (11) and the approximation (10) cannot be solved for $\partial/\partial r (r \ddot{u})$ (with $\ddot{u} \approx 0$), for the balance of p' against $\rho \phi'$ is delicate and the two approximations are inadequate. But multiplying equation (10) by a constant λ and combining equations (10), (11) and (12) (with $\ddot{u} = 0$) gives $|\lambda - 1| \approx (\text{density scale height}/r) \approx 0.005$. Thus equation (10) is quite accurate.

After integrating equation (14) we define

$$\tilde{V} = -\frac{\partial}{\partial r}(rv)/r\rho = -(l-1)\xi_u[g(r-r_1)-p(r_1)/\rho]/r^2 \quad (15)$$

The θ and ϕ components of the transverse poloidal acceleration are respectively¹² $\partial\tilde{V}/\partial\theta$ and $(\partial\tilde{V}/\partial\phi)/\sin\theta$. The second term in the bracket is dominant. The spherical harmonic angular dependence of ϕ' is also present in ξ_u and in V . Thus the shape of the surface distortion (ξ_u) determines the transverse fluid velocity from equation (15).

Even if there were a non-zero toroidal part of the circulation current the 'whole-disk average' of the Doppler velocity would preclude observing it. The gradient and poloidal parts of the velocity can be observed, but only the poloidal transverse part of a 13-day period can be significant. Choosing the line of sight as the axis of the spherical coordinate system, only the $m=0$ harmonics (Legendre polynomials) can be observed.

Neglecting limb darkening effects, the symmetry of the whole disk average imposes other restrictions on the results. Except for $l=2$, spherical harmonics of even degree are unobservable in the whole disk average and $l=2$ yields only the second harmonic of the core rotation frequency. The $l=1$ degree cannot occur in ϕ' . The $l=3$ spherical harmonics yield the fundamental and the third harmonic of the core rotation frequency. The $l=3$, $m=3$ mode (referred to the solar axis) is the strongest source of the fundamental period.

If the above interpretation of the data is correct, the $l=3$ harmonic is probably the source of the circulation current. A detailed comparison with the 1966 observations is not useful, for the telescope was incapable of detecting odd degree spherical harmonics.

Calculating the θ component of the velocity for $l=3$ from equation (15) and integrating over the solar disk gives

$$A = \frac{Tap(r_1)}{2\pi r^2 \rho} \quad (16)$$

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for the velocity amplitude where $\xi_u = aP_3(\cos\theta)$ and T is the core rotational period. Assuming $l=3$, $\Delta r = r - r_1 = 5 \times 10^7$ cm, $p(r_1) = 5 \times 10^5$, $\rho = 4 \times 10^{-8}$ and $a = 10^6$ cm gives 440 cm s^{-1} for A . The value of the coefficient a was chosen arbitrarily but it is reasonable.

If the above interpretation is correct there is a surprising conclusion. The above value (a) for the odd spherical harmonic distortion ($l=3$) is fairly large, of the same order of magnitude as $l=2$ and $l=4$ distortions⁴. This suggests that if there is a strong magnetic field buried in the solar core, it may be quite complex in form.

The centrifugal distortion due to the rotation of the surface should also drive an Eddington-Sweet current. In this case the flow is from the Equator to the pole and the velocity varies as $\sin 2\theta$. The velocity is limited by turbulent viscosity which is not easily estimated. Also this flow may be dominated by other effects.

Durrant and Schröter¹³ have suggested that the Claverie *et al.* 13-day period may be a spectroscopic artefact associated with the rotating magnetically active zones on the Sun's surface. From the plage maps for the same observational period used by Claverie *et al.*, they compute a function (here $D(t)$) which is similar in appearance to the Claverie function. Their effect should exist, but the magnitude is uncertain. I find that a simple sinusoid (Fig. 1) provides a substantially better (robust) fit to the velocity data than does the D function. Also, adding the $D(t)$ term to the sinusoidal fit does not significantly improve the fit. Also, the $D(t)$ fits better if it is phase advanced by 2 days (55°), $D \rightarrow D(t+2)$. I conclude that the Durrant-Schröter effect should exist but that it is probably weak, and perhaps negligible in the Claverie *et al.* data. Edmunds and Gough have independently considered the same artefact. From a study of line formation either side of the potassium line they conclude that the magnitude of the effect is approximately right to account for the observations¹⁴.

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Redox state of Earth's upper mantle from kimberlitic ilmenites

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Temperatures and oxygen fugacities are reported on discrete ilmenite nodules in kimberlites from West Africa which demonstrate that the source region in the upper mantle is moderately oxidized, consistent with other nodule suites in kimberlites from southern Africa and the United States. A model is presented for a variety of tectonic settings, proposing that the upper mantle is profiled in redox potential, oxidized in the fertile asthenosphere but reduced in the depleted lithosphere.

VOLATILES and the oxidation-reduction environment exerted by gas species in the system C-O-H-S are considered to have had profound effects on planetary and atmosphere formation¹ and are critical to petrogenetic models for the generation of magmas within the Earth's upper mantle². The compositions of volatiles in this source region influences degrees of partial melting, the composition of the melt, mass fractionation, liquid lines of descent, valency states of elements, and elemental partitioning coefficients between liquids and solids. Gas samp-

ling of active subaerial volcanoes^{2,3} and analyses of trapped volatiles in quenched ocean floor glasses have provided a wealth of new data on volatiles being generated at present^{2,4-7}. Sampling of the upper mantle through nodule suites in alkali basalts and kimberlites have received less attention, but the realization that these materials provide direct information on the volatile budget of the mantle and on the redox state of subcrustal regions is rapidly being recognized. These data have far-reaching implications ranging from mantle metallogenesis^{8,9}, to the origin of

diamonds¹⁰, and the sources of deep-seated magnetic anomalies^{11,12}. Although the study of mantle nodules to determine the redox state of the upper mantle is in its infancy there has been considerable debate on whether it is reduced^{13,14} and is equivalent to equilibrium conditions defined by iron-wüstite (IW), or on whether it is more oxidized¹⁵⁻¹⁷ and, hence, is more appropriately defined by equilibria consistent with magnetite-wüstite (MW) or fayalite-magnetite-quartz (FMQ). In the extreme case at magmatic temperatures (1,200 °C), these estimates differ by approximately five orders of magnitude in oxygen fugacity (f_{O_2}). Translated, this difference is similar to the differences between the oxidation states of lunar and terrestrial basalts, a disparity that is unreasonably large. In this study we conclude that the source region is compatible with WM-FMQ and possible explanations for the diversity of redox states reported include the ages of the mantle being sampled and whether that portion of the mantle is mature and depleted, or juvenile and enriched in volatiles.

Three methods have been used to estimate the redox condition of the upper mantle, and all rely on the variations of iron: oxygen ratios in minerals and on the magnitude of Fe^{2+}/Fe^{3+} concentrations. Thermodynamic techniques rely on theoretical solution models for coexisting minerals^{16,17}. In the intrinsic f_{O_2} method, furnace gas mixtures (CO or $CO_2 + H_2$) are adjusted as a function of temperature (T) to match the redox state of the sample^{13,14}. In the third technique, exchange equilibria between Fe and Ti , and between Fe^{2+} and Fe^{3+} , define unique solutions for T and f_{O_2} in co-equilibrated members of the ilmenite and spinel mineral series¹⁸. The present study used the last of these methods and it has also been used to estimate the redox states of planetary basalts¹⁵. A critical factor in this application is that the mineral pair is demonstrated to have co-equilibrated, a situation that is difficult to prove because ilmenite and spinel members are typically discrete constituents in igneous and metamorphic rocks. In the assemblages described here, however, the two components are in the grain boundary contact, and ionic exchange between ilmenite and spinel, in response to external environmental conditions, is reassured.

Samples and analytical techniques

One aspect of an extensive geochemical programme on the characterization of the West African kimberlites and their associated upper-mantle derived nodule suites^{19,20}, has been a detailed investigation of the oxide minerals for exploration purposes, magnetic properties and because of their sensitivity to modification in oxidizing or reducing conditions. Ilmenite occurs in great abundance in the Cretaceous kimberlite fields of Liberia, Sierra Leone and Guinea, and ilmenite is part of the discrete nodule suite typical of other kimberlite provinces, but debated in terms of their precise depths of origin²¹. That ilmenite nodules in kimberlites originate in the upper mantle is indisputable. This is borne out by diamond inclusion studies^{22,23}, by garnet and pyroxene intergrowths^{21,23}, and by high-pressure, high-temperature phase-equilibria experiments^{24,25}, that all include ilmenite as a component.

The ilmenites we have examined are typically 1–2.5 cm in diameter with some as large as 15 cm. Spherical or oblate spheroidal in shape, the ilmenites are free of surface weathering but occasionally show some reaction with the enclosing kimberlite, manifested by very thin veneers of late stage perovskite and associated calcite. Examined in reflected light and under oil-immersion microscopy, a large number of ilmenites are observed to contain parallel, oriented lamellae (20–100 μm in width) of spinel (titanomagnetite) that are crystallographically controlled along {0001} rhombohedral ilmenite planes. Among these samples are two contrasting types of assemblages. In some ilmenites from the Koidu Complex, Sierra Leone, exsolution of ilmenite lamellae within the host ilmenite has taken place, and this process is followed, or accompanied by subsolidus equilibration that has also formed spinel (Fig. 1a). Rounded

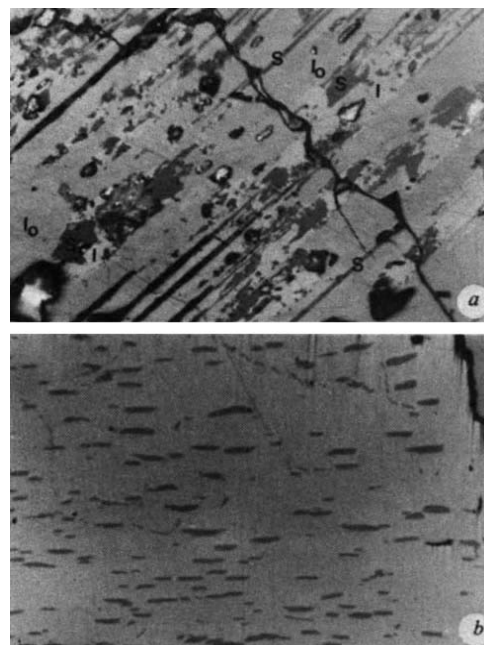


Fig. 1 Reflected light photomicrographs taken under oil-immersion objectives. The width of each plate is 0.475 mm. The field of view shown in *a* is typical of assemblages observed in ilmenite megacrysts from Koidu, Sierra Leone. The light grey, host ilmenite is the original (I_0) ilmenite that has undergone partial re-equilibration to ilmenite (I) + spinel. The bright and highly reflective areas are carbonate inclusions or minor sulphides. In *b* the dark oriented lamellae are spinel in an ilmenite host typical of megacrysts from Liberia, and the Antoschka kimberlite pipe in Guinea.

or curvilinear liquid immiscible sulphide (pyrite, pyrrhotite, pentlandite, chalcopyrite) and carbonate inclusions are present, and the spinels are interpreted to derive from subsolidus reduction. In other ilmenites from Koidu, and in all of the ilmenites from Liberia and Guinea (Antoschka pipe), the textural relations consist simply of parallel sets of spinel lenses elongated along {0001} ilmenite planes (Fig. 1b). These assemblages also result from reequilibration, and it is assumed, based on experimental studies^{18,26}, that solid state reconstitution has taken place in reducing conditions, transforming homogeneous ilmenite into a compositionally modified ilmenite + spinel. This textural and mineral relation is described as reduction exsolution. Although we cannot demonstrate that reduction took place in the source region, this appears most probable, and the process was deuteric modification with prolonged cooling. We can confidently eliminate the irruptive kimberlite event as the reduction agent, because of exsolution (*sensu stricto*) of ilmenite and the rarity of external reactions.

Compositions of coexisting spinel and ilmenites were determined by electron microbeam techniques using an ETEC Autoprobe, natural and synthetic mineral standards, and conventional correction procedures^{27,28}, with standard operating conditions (15 kV and 0.03 nA specimen current). Iron(II) concentrations were calculated assuming ideal analyses and mineral stoichiometry. Reliable analyses were only possible on the coarsest intergrowths, and only those data for which repeated and reproducible compositions were obtained are considered. Averages presented are a minimum of 10 analyses.

Upper-mantle derived oxides are commonly enriched in MgO , MnO , Cr_2O_3 , and Al_2O_3 , and in the classical application of Buddington and Lindsley's¹⁸ Fe - Ti oxide geothermometer and oxygen geobarometer, T and f_{O_2} estimates were only possible in assemblages having negligible contents of elements other than Fe - Ti - O . A revised thermodynamic model²⁹, and a method of treating these other components has been proposed³⁰, and the T and f_{O_2} of coexisting oxides in kimberlites can now be obtained using the equations: $X_{ilm} =$

$(\text{Mg} + 0.5\text{Al} + \text{Fe}^{2+}) / (0.5\text{Fe}^{3+} + \text{Mg} + 0.5\text{Al} + \text{Fe}^{2+})$, and $X_{\text{Usp}} = (\text{Ti} - 0.5\text{Mn}) / (\text{Ti} - 0.5\text{Mn} + 0.5\text{Fe}^{3+})$, where X_{ilm} and X_{Usp} are the mole fractions of ilmenite (in ilmenite solid solution) and ulvöspinel (Fe_2TiO_4) in spinel solid solution, respectively. Uncertainties in T and f_{O_2} (excluding Cr_2O_3) by this method are approximately 40–80 °C and 0.5–1.0 log unit f_{O_2} , assuming $\pm 1\%$ uncertainties in spinel and ilmenite compositions²⁹. We have modified the above equations, treating Cr_2O_3 in a manner similar to Al_2O_3 and although the precise effect of these oxides on T and f_{O_2} remains unknown, we estimate that the values are still within the range ~ 100 °C and ~ 1 log unit f_{O_2} . These estimates are corroborated by low $\text{Cr}_2\text{O}_3 + \text{Al}_2\text{O}_3$ mineral pairs and by independent experimental data³¹ in which we consider ilmenite alone in the system FeTiO_3 (ilmenite)– MgTiO_3 (geikielite)– Fe_2O_3 (haematite) as a function of f_{O_2} at constant T (1,300 °C). The data are also consistent with the estimates obtained on kimberlitic ilmenites from southern Africa and the United States, by thermodynamic treatment¹⁷, and using the intrinsic f_{O_2} method³².

Results

The compositions of 17 ilmenite–spinel pairs (five from Koidu and six each from Liberia and Antoschka) are expressed as major element, combined divalent ($\text{FeO} + \text{MgO} + \text{MnO}$), trivalent ($\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3 + \text{Cr}_2\text{O}_3$), and tetravalent (TiO_2) oxides in Fig. 2. Spinel from Koidu lie on or very close to the join Fe_2TiO_4 – R_3O_4 and all remaining spinels, with one exception, lie along joins slightly enriched in TiO_2 , a reflection of their MgO concentrations. Partitioning of MgO is preferential into ilmenite; hence, all of the ilmenite data fall between FeTiO_3 and MgTiO_3 . An approximate evaluation of these data, in terms of f_{O_2} , may be obtained from the inset in Fig. 2, based on the relative positions of coexisting compositions, and the slopes of tie-lines between ilmenite and spinel solid solution joins¹⁸. The more enriched Fe^{3+} samples (Koidu) are more oxidized (NNO), and those with higher Fe^{2+} contents (Liberia) are relatively more reduced (WM).

Absolute T and f_{O_2} s obtained from these data are illustrated in Fig. 3, and four of the standard T – f_{O_2} buffers are included for reference. These are IW, WM, FMQ, and MnO – Mn_3O_4 . Nine of the 17 oxide pairs lie, within experimental limits, along the FMQ buffer; five pairs are more oxidized than FMQ; and three are more reduced than FMQ. Because the assemblages have undergone reduction–equilibration, original single phase

ilmenites must have been stable at f_{O_2} s above those shown for each of the coexisting oxide pairs. Also included in Fig. 3 are data from the Precambrian Premier kimberlite³³, the Cretaceous Oka carbonatite³⁴, the Benfontein kimberlite–carbonatite sills³⁵ (also Cretaceous in age), experimental data on ilmenite from the Monastery (Cretaceous) kimberlite²⁶, along with data from peridotites enclosed in Tertiary alkali basalts¹³. The most striking feature of the diagram is that all data, with the exception of the peridotites, are more oxidized than MW. The peridotites bracket IW, and these data, in common with other equally reduced values on olivine in basalts³⁵, were made using the intrinsic f_{O_2} , Zr–electrolytic cell technique^{36,37}. Kimberlitic ilmenites, however, yield values coincident with FMQ³². Oxygen fugacity increases with T but also increases with pressure, and in Fig. 4 the f_{O_2} buffers are corrected to $P = 30$ kbar to illustrate this effect. The extent to which the ilmenite–spinel data are modified at high P is unknown, but the sense of the correction is towards higher values of f_{O_2} . Both the oxide data and the buffer curves will be synchronously displaced with increasing P . However, maintaining the oxide data as uncorrected values, because the correction is considered to be small¹⁸, and assuming 30 kbar, the Koidu data approach FMQ, the Oka and three of the Liberian compositions are equivalent to WM, one is more oxidized and the remainder more reduced. The Antoschka data lie ~ 1 log unit below WM. Because the precise pressures are not known the relative f_{O_2} s are clearly only estimates.

Kimberlitic ilmenites are dominated by solid solutions among three endmember components (FeTiO_3 – MgTiO_3 – Fe_2O_3) and because the oxidation state of iron in two of these endmembers is either exclusively Fe^{2+} or Fe^{3+} (that is ilmenite and haematite, respectively), the stability of any composition within the ilmenite ternary must be f_{O_2} dependent. This f_{O_2} dependency is illustrated in Fig. 5 based on experimental data³¹ at 1,300 °C. The West Africa data are shown together with published data on the compositions of ilmenites from kimberlites, worldwide^{23,38,39}. Most compositions lie within the range of f_{O_2} between 10^{-6} and 10^{-9} atm. The WM buffer at 1,300 °C and 30 kbar, has an $f_{\text{O}_2} = 10^{-6}$ atm. A more appropriate buffer for upper mantle conditions has been proposed by Eggler and Baker¹⁶, which is defined as EMOG, and is the intersection of

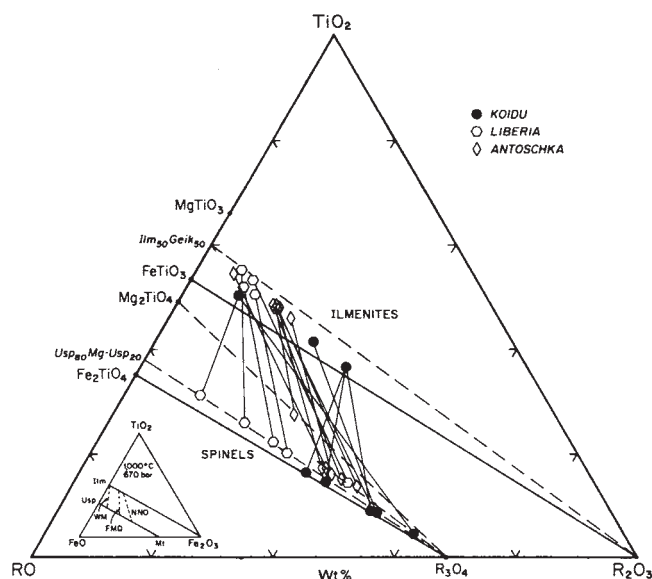


Fig. 2 Electron microbeam analyses of coexisting spinel and ilmenite compositions as a function of RO ($\text{FeO} + \text{MgO} + \text{MnO}$), R_2O_3 ($\text{Fe}_2\text{O}_3 + \text{Cr}_2\text{O}_3 + \text{Al}_2\text{O}_3$), TiO_2 .

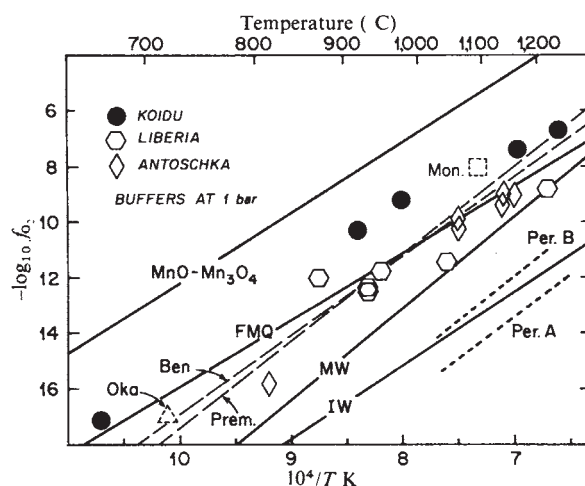


Fig. 3 Temperature–oxygen fugacity relations for coexisting ilmenite–spinel pairs from West Africa (Liberia; Antoschka–Guinea; and Koidu–Sierra Leone) determined according to the methods described in refs 18, 29, 30. Other data shown are for the Premier (Prem.) kimberlite³³, the Oka carbonatite³⁴, the Benfontein (Ben) kimberlite–carbonatite sills³⁵, the Monastery kimberlite²⁶, and peridotites (Per. A and Per. B) in alkali basalts^{13,46}. The solid lines are experimental buffers MnO – Mn_3O_4 (ref. 52); FMQ (fayalite–magnetite–quartz)⁵³; MW (magnetite–wüstite) and IW (iron–wüstite) are from ref. 54. The salient feature to be noted is that the peridotite data are considerably more reduced than those from kimberlites and carbonatites.

two univariant reactions: (1) enstatite + magnesite + carbon = forsterite + vapour; with (2) magnetite + carbon = wüstite + vapour. This intersection is at 29.6 kbar, 10^{-7} atm and 1,230 °C. At lower T , EMOG lies between WM and FMQ, but above 1,230 °C at high pressures, graphite is replaced by diamond and EMOD is ~ 1 log unit more reduced than WM. If the equilibrated data in Figs 3 and 4 are extrapolated to 1,300 °C, parallel to the FMQ buffer, the range in resultant f_{O_2} is $10^{-5.6}$ to 10^{-9} atm. This corresponds almost exactly to EMOG, WM, and the range of compositions in Fig. 5, noting that it is only the ilmenite component of the oxide pair that is considered in this treatment. This estimate in f_{O_2} is consistent with the thermodynamic approach for coequilibrated oxides and silicates from a variety of upper-mantle derived nodules^{17,40}, and with

intrinsic f_{O_2} data on kimberlitic ilmenite³². The estimate is also consistent with the oxygen fugacities of continental basalts in which, $\approx 90\%$ of the data lie between FMQ and WM^{2,38}.

Discussion

It has been argued⁴¹ that the presence of graphite or carbon polymorphs, or the occurrences of CH_4 and CO in ocean floor and upper-mantle derived nodules mandates that the mantle be reduced. We consider that these assumptions are erroneous: (1) because the abundances of reduced carbon species are negligible, amounting in most cases to concentrations of ≈ 0.5 wt% in media that are dominantly $CO_2 + H_2O$ (ref. 2); (2) graphite is stable over an enormous range of f_{O_2} and temperature conditions⁴²; (3) graphite or diamond formation is possible by either oxidation ($CH_4 \rightleftharpoons C + 2H_2$) or reduction ($CO_2 \rightleftharpoons C + O_2$), and diamond is in equilibrium with $CO_2 + CH_4 \rightleftharpoons 2C + 2H_2O$ (ref. 10), so that extremely reducing conditions are not necessarily a prerequisite to carbon precipitation; (4) whether carbon forms from a single (such as $CO \rightleftharpoons C + \frac{1}{2}O_2$) or a multiple series of reactions (such as $CO_2 \rightleftharpoons CO + \frac{1}{2}O_2$), in which CO is an intermediate product, typically cannot be determined and hence the conditions of f_{O_2} are indeterminant; (5) high-pressure equilibria in the system C-H-O show that CO is never a major C-bearing species in the vapour¹⁰; (6) methane, however, may be present in mantle conditions¹⁰ although evidence to the contrary suggests that the equilibria are more appropriately defined by CO-CO₂ (refs 16, 17). In a broader context we note that carbon + magnetite are typical of chondritic meteorites and some classes of metamorphic rocks, and that these are relatively oxidized.

Degassing of the upper mantle has taken place with time and an inevitable consequence of degassing is that the redox state of the mantle must also have changed with the continuous loss of volatiles. The question is whether the mantle has become more reduced or more oxidized, or whether the condition is steady state. The latter assumption is that volatile loss through volcanism is offset by ocean floor subduction of hydrous materials. Present-day basaltic volcanism and the Cretaceous kimberlite and carbonatitic suites collectively imply mantle redox conditions between FMQ and WM. However, we offer two tantalizing and perhaps crucial pieces of data on the redox state of the early mantle. These are that Ni-poor metallic Fe has been identified as an inclusion in diamond⁴³ and that the ages of diamonds, regardless of the time of kimberlite intrusion are Archaean⁴⁴. Considerably more data are required but we propose that the early mantle may well have been reduced (for example, $\leq WM$) and that some nodule suites brought to the surface in kimberlites and alkali basalts reflect this primitive condition: even though the bulk content of volatiles would have been much greater, the relative proportions of reduced gas species would also have been greater. With time, however, the mantle has become progressively oxidized, resulting perhaps largely from the preferred loss of hydrogen and carbon. Another important factor is that if the upper mantle is geochemically layered, that is with an upper depleted zone overlying a more fertile region⁴⁵, it is likely that the redox condition of each component will differ. Depleted zones result from extensive volcanism, so that the regional, stratigraphical and age of the mantle zone being sampled in nodules must be considered. In mature mantles underlying cratonic regions of the Earth, a mixed sampling of oxidized and reduced components such as those present in kimberlites will differ from more fertile mantles actively undergoing depletion. Thus, depleted and refractory upper portions of mature mantle are likely to be relatively reduced. Similarly high level but juvenile and fertile regions of the mantle, such as those underlying the ocean floors, are relatively enriched in volatile constituents and are, therefore, more oxidized. The plausible ranges of f_{O_2} are WM and FMQ, respectively.

The mantle (and crustal) f_{O_2} model that we propose is illustrated in Fig. 6. Five typical tectonic cross-sections are outlined

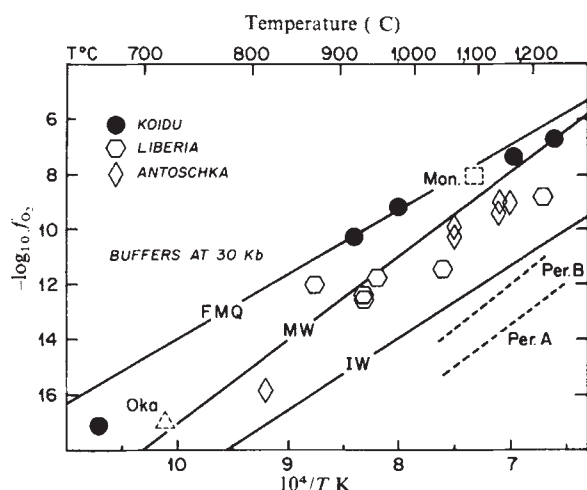


Fig. 4 Pressure corrected f_{O_2} buffers to 30 kbar, illustrating the effect of P on the relative f_{O_2} for the 1 bar data shown in Fig. 3. No correction has been applied to the mineral data.

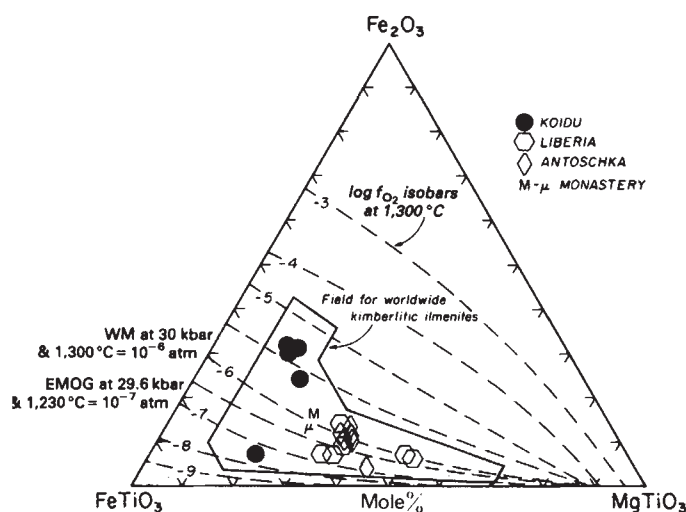
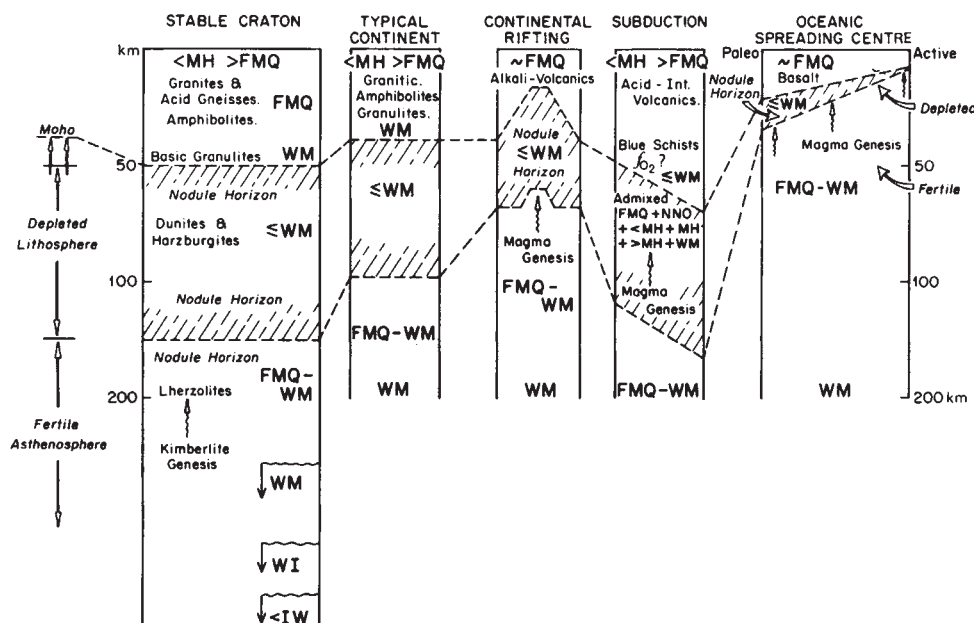


Fig. 5 Ilmenite compositions from West Africa (see legend to Fig. 2) plotted as a function of Fe_2O_3 (haematite), $FeTiO_3$ (ilmenite) and $MgTiO_3$ (geikielite). M and μ refer to an ilmenite starting composition, and to an experimentally equilibrated composition, respectively from the Monastery kimberlite²⁶. The generalized field for kimberlitic ilmenites worldwide are from a compilation of data cited in refs 23, 38, 39. Oxygen fugacity isobars are from ref. 31; WM, wüstite-magnetite⁵⁴ and EMOG (see text for explanation) is from ref. 16. There is a large solvus in the ternary extending from the Fe_2O_3 - $MgTiO_3$ sideline towards $FeTiO_3$ which has been omitted for clarity. It is considered that the distribution of kimberlitic ilmenite compositions is influenced by this ternary solvus and by the f_{O_2} environment of formation²⁰.

Fig. 6 Selected but typical cross-sections through the crust and upper mantle illustrating the distribution of rock types and profiles of oxygen fugacities. The highest f_{O_2} is represented by conditions less than magnetite-haematite (MH) equilibria. With increasing reduction the equilibria are NNO (Ni-NiO), FMQ (fayalite-magnetite-quartz), WM (wüstite-magnetite), and IW (iron-wüstite). The perched and depleted lithospheric layer results from sustained volcanism and varies in thickness as a function of mantle maturity and tectonic setting. Nodules from the depleted lithosphere arise from craton, rift, and palaeo-spreading environments. Nodules from the fertile asthenosphere are most common in kimberlites but are also present in smaller concentrations in continental rift settings, and are only rarely encountered in palaeo-spreading terrains. This distribution is related to the depth of magma generation which is illustrated. In the text we conclude that the reduced state of the mantle that is inferred by some^{13,14,46} results from selective sampling of depleted lithospheres. The lower portion of the upper mantle, in the fertile asthenospheres is oxidized and conditions are at FMQ ranging down to WM, to WI, and to iron at the lower mantle-outer core interface.



and the oxygen fugacities we have designated are from data presented here and cited earlier^{2,15-17,26,32-35,40}. For the uppermost regions of each cross-section, oxygen fugacities range from below magnetite-haematite (MH) to FMQ, and representative examples are cratonic crust to ocean floor basalts, respectively. Oxygen fugacities decrease within the crust and we propose that a redox discontinuity is present at the Moho, transforming from FMQ to WM. Lower crustal granulites are most typically feldspathic, with almandine-rich garnet and Na-Fe³⁺-bearing clinopyroxene; hydrous minerals, such as mica and amphibole may also be present. Hence these rocks are more oxidized than the underlying and depleted dunites and harzburgites. Although we suggest that the depleted lithosphere is at WM, it could conceivably be lower than WM but not much less, because spinels in these rocks are iron(III)-bearing³⁸. The fertile asthenosphere, below the depleted lithosphere, signals a return to more oxidizing conditions (between FMQ and WM), represented by lherzolites and the genesis of highly volatile kimberlites and carbonatites. With increasing depth the redox state is WM, followed by WI and in the lower mantle-outer core boundary by iron. This model is applicable to four of the five cross-sections (Fig. 6), with the sub-Moho region of the subduction profile being more complex, resulting from admixtures of various redox components. Volcanism arising from these zones are mafic but also acid-intermediate in composition and are more highly oxidized than basalts at spreading axes or undersaturated volcanics in continental rift systems. We draw attention to two final points: (1) the depleted lithosphere varies enormously in thickness; and (2) only three tectonic settings give rise to mantle nodule suites: cratonic, continental rifts, and palaeo-spreading centres. The common factor is that sustained basaltic volcanism has produced a depleted lithospheric mantle horizon. Magma genesis in cratonic settings is usually at depths >150 km, and hence both fertile lherzolites and depleted dunites and harzburgites are entrained on eruption. On the other hand, in alkali-rift environments, magmas are generated at much higher levels and the opportunity for sampling fertile lherzolites is diminished. Hence, these volcanics most commonly contain a depleted and refractory nodule suite. Melting takes place in the redox zone of WM-FMQ and the resulting volcanics are FMQ (Fig. 6), similar to those of kimberlites and carbonatites (Fig. 3). Oxygen fugacity assessments, therefore, which are based on mantle-derived suites will depend on

whether the nodules arise from depleted lithospheric mantle or from the fertile asthenosphere. Herein may lie the source of the disparity reported in mantle redox conditions.

Arculus and Delano⁴⁶ have presented comprehensive and convincing arguments for the oxygen fugacity for the early Earth that includes core formation and the origin of the atmosphere. We are in general agreement with most aspects of their model but we differ in interpretation. They conclude that the mantle is buffered by IW and that the observed state of effusive volcanism at FMQ results from oxidation during upward migration of magma. The premise for IW is based on data derived exclusively from the electrolytic cell technique, and an explanation to reconcile these low f_{O_2} values is auto-reduction from particulate graphite⁴⁷, fluid inclusions comprising reduced carbon species (for example, CH₄ or CO), or some property related to thermal relaxation of high-pressure minerals when heated to elevated temperatures at 1 atm. However, the most probable reason is that the data are not an artefact of the method used but rather that the peridotites they measured (Fig. 3) are all highly depleted continental rift nodules. Their model is, therefore, based only on the sandwiched and depleted lithosphere layer between the Moho and the fertile asthenosphere. They have not considered the latter.

Iron(III) and (II) equilibria in natural silicate liquids⁴⁸, and the pressure dependency of oxygen fugacity in magmas, show that f_{O_2} increases with increasing P (as in Fig. 4) and decreasing silica activity⁴⁹, which implies that the f_{O_2} of magmas (specifically mafic) at depth is greater than that at the surface, assuming isochemical ascent. The situation is clearly not this simple based on the speciation of iron (Fe²⁺/Fe³⁺), and iron-oxygen coordination in silicate melts as functions of P , T , f_{O_2} and bulk composition^{50,51}. In alkali-rich systems (such as NaAlSi₃O₈-NaFe³⁺Si₂O₆, or Na₂O-Fe²⁺O-SiO₂), the ratio of Fe³⁺/total iron decreases systematically from 1 to 10 kbar, plateaus between 10 and 20 kbar, and increases again from 20 to 30 kbar. Taken in a broader context, and recognizing that the experimental compositions are not directly applicable, it is nonetheless noteworthy, that the parabolic behaviour of Fe³⁺ as a function of P is consistent with the reduced state at low pressures determined by Arculus and Delano⁴⁶, and supports the data we present at higher pressures in kimberlites. The interval between 10 and 20 kbar is relatively reduced and corresponds approximately to that horizon within the litho-

sphere that we propose is similarly depleted in volatiles (Fig. 6), and hence, is more reduced.

Conclusions

Upper-mantle derived ilmenite nodule suites from kimberlites in West Africa are moderately oxidized and lie within redox states consistent with the determinations obtained on other nodule suites in kimberlites by thermodynamic and intrinsic f_{O_2} methods. These data contrast with lower pressure nodule suites in peridotites from alkali basalts which yield highly reduced values. A single all-encompassing redox state for the mantle cannot be uniquely inferred. Oxidation and reduction of the Earth's upper mantle has evolved with time, and an expression of this evolution is provided by the relative thicknesses of redox layers ranging from those in cratonic regimes to those at active oceanic spreading centres (Fig. 6). Crustal setting and tectonic style, along with nodules and volcanism provide the important clues to mantle redox conditions. Mantle source regions for all but subduction-related volcanism is WM-FMQ. Subducted ter-

rains are more oxidized and lie between NNO (Ni-NiO) and MH. A knowledge of the stratigraphical position that these samples represent is fundamental to resolving the redox state of the upper mantle. The issue of mantle fugacities will not be settled until more precise data are available permitting specific sample horizons to be definitively located. Cretaceous kimberlites and carbonatites were unquestionably generated in an oxidized upper mantle below a depleted lithosphere. However, whether extreme depletion of such a zone could have led to IW⁴⁶ remains uncertain and problematic. A perched reduced zone appears probable in our model but an IW condition for the entire upper mantle appears unlikely.

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Structure of mouse kallikrein gene family suggests a role in specific processing of biologically active peptides

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The glandular kallikrein gene family comprises 25–30 highly homologous genes that encode specific proteases involved in the processing of biologically active peptides. In the mouse all the members of this family are closely linked on chromosome 7. The 9.5-kilobase nucleotide sequence of a mouse genomic clone contains one complete kallikrein gene (mGK-1), which is expressed in the male mouse submaxillary gland, and the 3' end of another (mGK-2). Differences in the coding potential of these genes and the amino acid sequences of other known kallikreins seem to be functionally related to the substrate specificity of the different enzymes.

BIOLOGICALLY active peptides are often synthesized as inactive precursors, requiring a highly specific cleavage event for activation. In some cases the processing event involves the removal of a 'pro' peptide by structurally similar but highly specific trypsin-like enzymes^{1,2}. These enzymes are members of the glandular kallikrein family, and form a distinct subgroup of the mammalian serine proteases³. Kallikreins are 25,000–40,000 molecular weight glycoproteins which exhibit a strict specificity in their proteolytic actions⁴. They are characterized by their ability to release vasoactive peptides (kinins) from kininogen *in vitro*⁵, although the observed kininogenase activity of different kallikreins is highly variable. Together with the demonstration that certain kallikreins are involved in the specific maturation of growth factors^{1,2}, this indicates that the true physiological role of these enzymes is often unrelated to their kininogenase activity. This diversity of physiological action is consistent with the presence of kallikrein activity in a wide variety of exocrine tissues³.

In the mouse one of the major sites of kallikrein synthesis is the male submaxillary gland where seven different kallikrein-like activities have been identified^{3,6}. Their presence in the intracellular granules of this tissue is correlated with the processing of various growth factors^{6,7}. The most thoroughly characterized of these kallikreins are epidermal growth factor binding protein (EGF-BP) and the γ -subunit of nerve growth factor (γ -NGF), which are responsible for the processing of epidermal growth factor and nerve growth factor, respectively^{1,2}. Although EGF-BP and γ -EGF exhibit a strict specificity for their particular substrate, they share extensive amino acid sequence homology and cross-react immunologically both with each other and with several other kallikreins also found in the submaxillary gland^{6,8,9}. The latter include γ -renin, which mimics the activity of the acid protease renin by cleaving angiotensinogen to angiotensin II *in vitro* (C. D. Bennett and M. Poe, personal communication) and β -NGF endopeptidase⁶.

The high level and variety of kallikrein activity in the submaxillary gland⁶, together with the presence of many growth factors such as thymocyte transforming factor, erythropoietin and two mesodermal growth factors^{10–13} as well as EGF and NGF, has led to the proposal of a physiological role for members of the kallikrein family in growth factor maturation^{3,6}.

Here we describe the cloning and initial characterization of a large gene family whose members encode very similar kallikreins. All the members of this family are localized on chromosome 7 in the mouse. Isolation and characterization of these genomic kallikrein clones has demonstrated that the kallikrein genes are frequently tightly linked and are highly conserved in their DNA sequence and structural organization. The limited variability in the coding regions of these genes largely involves amino acid residues implicated in substrate specificity.

Kallikrein genes localized on chromosome 7

We have described previously the isolation and characterization of a mouse submaxillary gland cDNA clone, pMK-1, which codes for a member of the kallikrein family¹⁴. Southern blot analysis of genomic DNA was used to determine whether pMK-1 would cross-react with other members of the mouse kallikrein gene family. Figure 1A shows the hybridization pattern obtained when the 500-base pair (bp) *Hind*III insert of this kallikrein cDNA clone was used as a probe on *Eco*RI-cleaved mouse DNA (lane 1) and rat DNA (lane 2). In both species there are multiple *Eco*RI fragments which hybridize to pMK-1. Differences in the intensity of hybridization of certain restriction fragments (for example, 2.4 and 6.7 kilobase (kb) in mouse DNA) are due to the conservation of particular restriction sites around several different genes (see below). As variation in both the levels and type of kallikrein gene expression in the submaxillary gland have been reported in several mouse strains¹⁵, it was important to determine whether there was variation in the kallikrein locus of these different

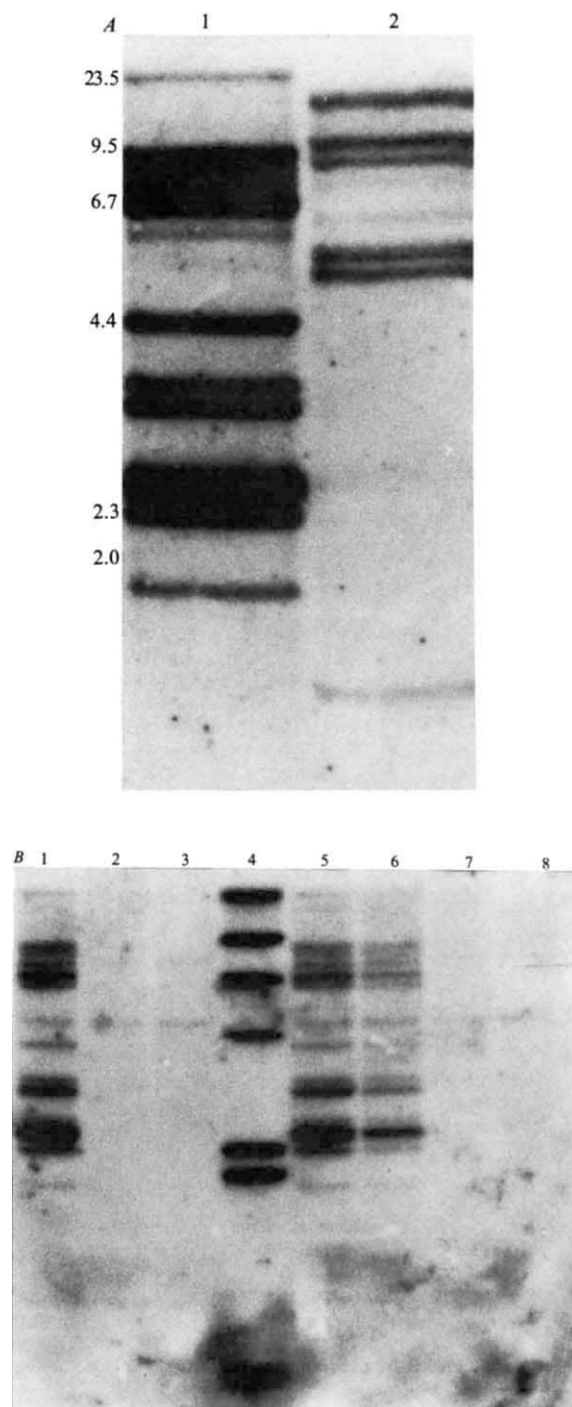


Fig. 1 A, Genomic blot analysis of the kallikrein genes in the mouse and rat. Genomic DNA (20 μ g) isolated from either Quackenbush mouse liver (lane 1) or Sprague-Dawley rat liver (lane 2) was digested to completion with the restriction endonuclease *Eco*RI, and electrophoresed in a 1% horizontal TAE (40 mM Tris, 20 mM Na acetate and 2 mM EDTA, pH 7.8) agarose gel at 40 V for 18 h. (The Quackenbush mouse is an inbred strain of Swiss Webster mice.) The DNA was then transferred to a nitrocellulose filter³³ and hybridized at 65 °C in 3 $\times$ SSC, with 10⁷ c.p.m. of the random primed³⁴ [α -³²P]dCTP-labelled kallikrein cDNA probe (pMK-1). The filter was washed in two changes of 2 $\times$ SSC at 65 °C, before autoradiography for 7 days at -70 °C in the presence of an intensifying screen. Numbers alongside the autoradiograph represent the size in kilobases of markers derived from a *Hind*III digest of λ cl857. B, Autoradiogram showing the results obtained with cell lines I-5, I-7B-4, I-3A-3, I-8-5, I-3A-2 and III-23 (tracks 1–3 and 5–7 respectively). Lane 8 is a Chinese hamster DNA control and lane 4 shows the *Hind*III λ markers described in A.

Table 1 Chromosomal localization of mouse kallikrein genes

Clone	Mouse chromosome																				Hybrid
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	
I-5*	+	+		+		+	+	+	+	+		+					+			+	+
I-7B-4	+	+								+				+	+			+	+		
I-3A-3	+	+		+					+				+		+			+			
I-8-5*		+		+		+	+			+		+			+				+		+
I-3A-2*		+		+			+	+				+	+		+	+		+	+		+
III-23		+ [†]	+					+				+		+				+		+	
I-8-2*	+	+		+		+	+	+		+		+	+		+	+			+		+
III-13*	+						+		+							+				+	+
III-14*	+			+			+								+	+			+	+	+
III-12					+	+						+	+	+			+			+	
CIV-12		+						+		+		+				+	+			+	
III-16-1*		+	+		+		+	+	+			+		+		+	+	+	+		+

Twelve different Chinese hamster-mouse hybrid cell lines were characterized with respect to their complement of mouse chromosomes; DNA isolated from each cell line was analysed for kallikrein-coding sequences as outlined in Fig. 1 legend.

* Hybrid cell lines which contained kallikrein sequences. Only chromosome 7 is common to all these hybrid lines.

† This clone contained a rearranged 2/18 metacentric chromosome, involving a translocation of chromosomal material of unknown origin to chromosome 2 at band 2D, with deletion of the normal chromosome material distal to band 2D¹⁶.

mice. The hybridization patterns observed with DNA isolated from two strains, BALB/c and C57BL/6 (data not shown), were indistinguishable from that observed for Quakenbush mouse DNA (Fig. 1A). This indicates a similar structural organization and kallikrein gene copy number in these mice.

The gene copy number in the rat seems to be similar to that of the mouse—about 10 *Eco*RI bands were observed (Fig. 1A, lane 2). Note that the extent of hybridization to the rat genome is significantly less than that in the mouse, suggesting that the mouse kallikrein genes are more similar to each other than to their counterparts in the rat genome.

The distribution of kallikrein genes within the mouse genome was investigated by using pMK-1 as a hybridization probe on DNA isolated from Chinese hamster-mouse hybrid cell lines. These cell lines were constructed by fusing V-79 Chinese hamster cells with cells derived from two feral strains of the mouse, *Mus musculus*¹⁶. The hybrid cell lines used to prepare DNA were karyotyped and analysed for various characteristic enzymes in order to assign definitively the particular mouse chromosomes present in each hybrid line. DNA prepared from 12 hybrid cell lines was cleaved with *Eco*RI and subjected to Southern blot analysis (see Fig. 1B and Table 1). Clearly, when hybridization to pMK-1 does occur, the complete set of *Eco*RI fragments is always present. The pattern observed with the hybrid DNA differs in only one position from that of Quakenbush DNA. This is probably due to a single restriction site polymorphism. The weak cross-reaction of pMK-1 with Chinese hamster sequences is shown in track 8 of Fig. 1B. The observation that chromosome 7 is the only chromosome common to the DNAs that hybridized demonstrates that all the kallikrein genes are present on this chromosome. This result also suggests that the members of this gene family may be closely linked.

Many different chromosomal loci have been described for esterase activities in the mouse¹⁷. One of these, termed the TAMase locus, is also (like the kallikrein genes) localized on chromosome 7 and expressed in the mouse submaxillary gland. However, Skow has shown that these enzymes are immunologically and electrophoretically distinct from the kallikreins¹⁸.

Kallikrein gene locus

To study the molecular organization of the kallikrein genes, we isolated mouse genomic clones from bacteriophage lambda

libraries by using pMK-1 as a hybridization probe. A random library of Quakenbush mouse DNA was constructed by ligating a partial *B*stI digest of genomic DNA into *B*amHI-cut Charon 28 (ref. 19). This library and a Charon 28 BALB/c mouse embryonic library were screened for kallikrein sequences. Of 30 unique clones that were isolated, eight have been analysed in detail (Fig. 2). Several clones were found to contain part, or all, of two kallikrein genes. One of these clones, designated λMSP-1, was chosen for detailed sequence and structural analysis. The two genes on λMSP-1 have the same direction of transcription; the first gene (*mGK-1*) is complete and consists of five exons and four introns, together spanning 4.5 kb. The 3' end of another gene (*mGK-2*) is located 3.7 kb upstream from the 5' end of *mGK-1*. In λMSP-1 no other kallikrein gene sequences were detected downstream from *mGK-1*. The exons comprising each of the two genes were mapped by using pMK-1 which cross-reacts with exons 3, 4 and 5, and also by using restriction fragments as probes on Northern blots of poly(A)⁺ RNA isolated from male mouse submaxillary gland. Figure 3A shows that restriction fragments used to probe RNA hybridized to a common mRNA size class of ~950 nucleotides. This result demonstrates homogeneity in the mRNA size of the many different kallikrein genes expressed in this tissue. The exon-intron boundaries were mapped precisely by detailed sequence analysis as described below. As well as λMSP-1, four other genomic clones were found to contain more than one kallikrein gene (Fig. 2). Only two of these (λMSP-6 and -8) contained common sequences and therefore represent overlapping cloned DNA fragments. The results of genomic blot analysis and the preliminary examination of all the λ clones (Fig. 2 and B.A.E., unpublished results) indicate that there are 25–30 different kallikrein genes in the mouse.

Primary genomic structures

A series of restriction fragments from λMSP-1 were subcloned into various sites of the plasmid pBR322. These overlapping subclones (see Fig. 3C), were used for detailed restriction mapping and as a source of DNA for sequence analysis. Almost 9,500 contiguous base pairs of the *mGK-1*, *mGK-2* gene region were determined by sequencing the majority of both strands. During this study we developed a rapid method of 'nonrandom' sequencing based on the generation of a series of deletions with the double-stranded exonuclease *Bal*31 (see Fig. 3 legend).

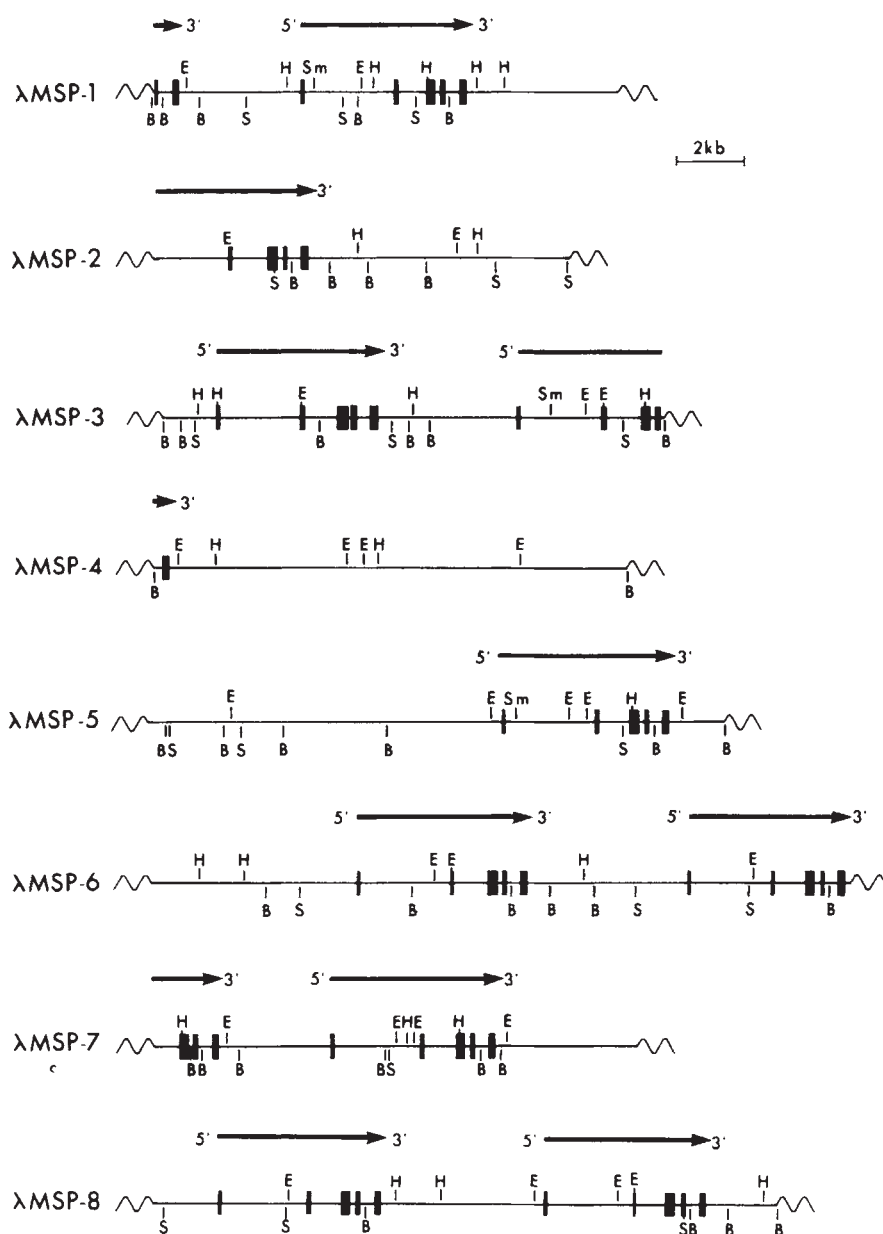


Fig. 2 Restriction maps of cloned DNA fragments containing mouse glandular kallikrein genes. The clones λ MSP1-8 were isolated from mouse genomic libraries using pMK-1 as a hybridization probe. A detailed map of clone λ MSP-1 (illustrated in Fig. 3) was constructed using data from restriction analysis, Northern blot hybridization, and direct nucleotide sequencing of exons (shown as vertical bars), intervening sequences and the spacer region between genes *mGK-1* and *mGK-2*. DNA fragments from λ MSP-1 corresponding to coding regions of the *mGK-1* gene were used as exon-specific probes in Southern blot analysis of restriction digests of clones λ MSP-2 to 8. Limited nucleotide sequencing of clones 2, 6 and 8 (data not shown) indicates that different kallikrein genes share common locations of the four intervening sequences. Therefore, the 12 genes are represented as having the same overall structural organization. Note, however, that the lengths of certain regions of DNA, particularly those of the first intervening sequence of each gene, are only approximate. Wavy lines represent the two arms of the Charon 28 vector, and arrows above each gene indicate the direction of transcription. Restriction sites: B, *Bam*HI; E, *Eco*RI; H, *Hind*III; S, *Sac*I; Sm, *Sma*I.

Figure 4 shows the complete sequence of the *mGK-1*, *mGK-2* gene region.

When the previously sequenced kallikrein cDNA pMK-1 was aligned with the sequence from *mGK-1* and *mGK-2* it was clear that neither *mGK-1* nor *mGK-2* represents the genomic pMK-1 sequence. However, they are each very closely related to pMK-1 with approximately 84% nucleotide sequence homology. Comparison of the coding potentials of *mGK-1* and *mGK-2* with the amino acid sequences of γ -NGF⁹, EGF-BP⁸ and γ -renin (Fig. 5) shows that these are all very similar but distinct kallikreins. Comparison of the nucleotide sequence of *mGK-1* with both the known protein sequences of the closely related γ -NGF, EGF-BP and γ -renin, as well as the cDNA sequence of pMK-1, facilitated the accurate mapping of exon-intron junctions. To determine whether the gene *mGK-1* was expressed in the submaxillary gland, a cDNA library was constructed from poly(A)⁺ mRNA. Sites for the restriction enzyme *Mbo*I are present in exon 1 (nucleotide 4,524) and exon 4 (nucleotide 8,353) of *mGK-1* (Fig. 4). To select for a *mGK-1* cDNA clone, the cDNA was cleaved with *Mbo*I and cloned into the *Bam*HI site of pBR322. A library of 2,500 clones was constructed and screened with an exon 1-specific probe from *mGK-1*. The complete 590-bp sequence of one of the positive

clones, pMK-4, was found to correspond exactly to the sequence of *mGK-1*, confirming our exon-intron assignment and demonstrating that the *mGK-1* gene is transcriptionally active in the submaxillary gland.

S₁ mapping experiments²⁰ were carried out in an attempt to map the site of transcription initiation. The results of these experiments were inconclusive because of apparent sequence heterogeneity in the 5' untranslated region of the different kallikrein mRNAs present in the submaxillary gland. To overcome this problem we performed a primer extension experiment²¹, using a 58-bp primer isolated from exon 2 of *mGK-1* (Fig. 3D). When submaxillary gland poly(A)⁺ mRNA was used as the template, we found two putative capping sites 36 and 42 bp upstream from the initiator ATG (Fig. 4). The sequence AGCTC was found at both potential capping sites.

There are four intervening sequences interrupting the coding region of the *mGK-1* gene. All four introns begin with a GT dinucleotide and end with AG, sequences thought to be necessary for correct RNA splicing²². The four introns are 2,418, 821, 96 and 372 bp long, respectively, and the corresponding five exons code for 15, 54, 95, 46 and 51 amino acids. The *mGK-2* gene exhibits a virtually identical 3' end structure to that of *mGK-1*; intron D occurs in exactly the same position

and separates exons 4 and 5 by 374 bp. While these exons from the respective genes show 82% conservation of sequence, the introns separating them are 79% homologous in nucleotide sequence when aligned for maximum homology.

The 3' untranslated regions of *mGK-1* and *mGK-2* (as predicted by analogy with the 3' untranslated region of *pMK-1*) are 45 and 48 bp long, respectively. The already striking nucleotide sequence homology in exons 4 and 5 is retained in the 3'

untranslated region. By allowing for a single 3-bp insertion in the *mGK-2* untranslated region, the respective sequences can be aligned to give 91% sequence homology. Both genes possess the putative poly(A) addition signal sequence, 5'-AATAAA-3' (ref. 23) located 19 bp from the 3' end of the mRNA (with respect to the *pMK-1* mRNA sequence).

The complete nucleotide sequence of the 3,752-bp spacer between *mGK-1* and *mGK-2* has been determined. This region should contain many of the sequences necessary for the expression of the *mGK-1* gene. The two putative capping sites are preceded by a 'Goldberg-Hogness' box²⁴ located 22 and 28 bp upstream. This sequence (5'-TTTAAA-3') is a variant of the consensus sequence (5'-TATAAA-3') thought to be important in determining the specificity of the initiation of RNA synthesis by RNA polymerase II²⁵. In addition, a 'CAT' box is located roughly 80 bp upstream from the cap sites²⁶. The most striking feature of the spacer is the presence of a 100-bp T+C-rich region 2,707 bp upstream from *mGK-1*. This region consists of a simple TC repeat interspersed by occasional runs of C residues (Fig. 4).

Kallikrein structure

The complete structure of the kallikrein gene *mGK-1* (Fig. 4) demonstrates that it encodes a preprokallikrein of 261 amino acids. The N-terminal sequence of the primary translation product consists of a proposed hydrophobic signal peptide 18 amino acids long. The presence of a signal peptide has not been previously demonstrated for the kallikreins but is expected on

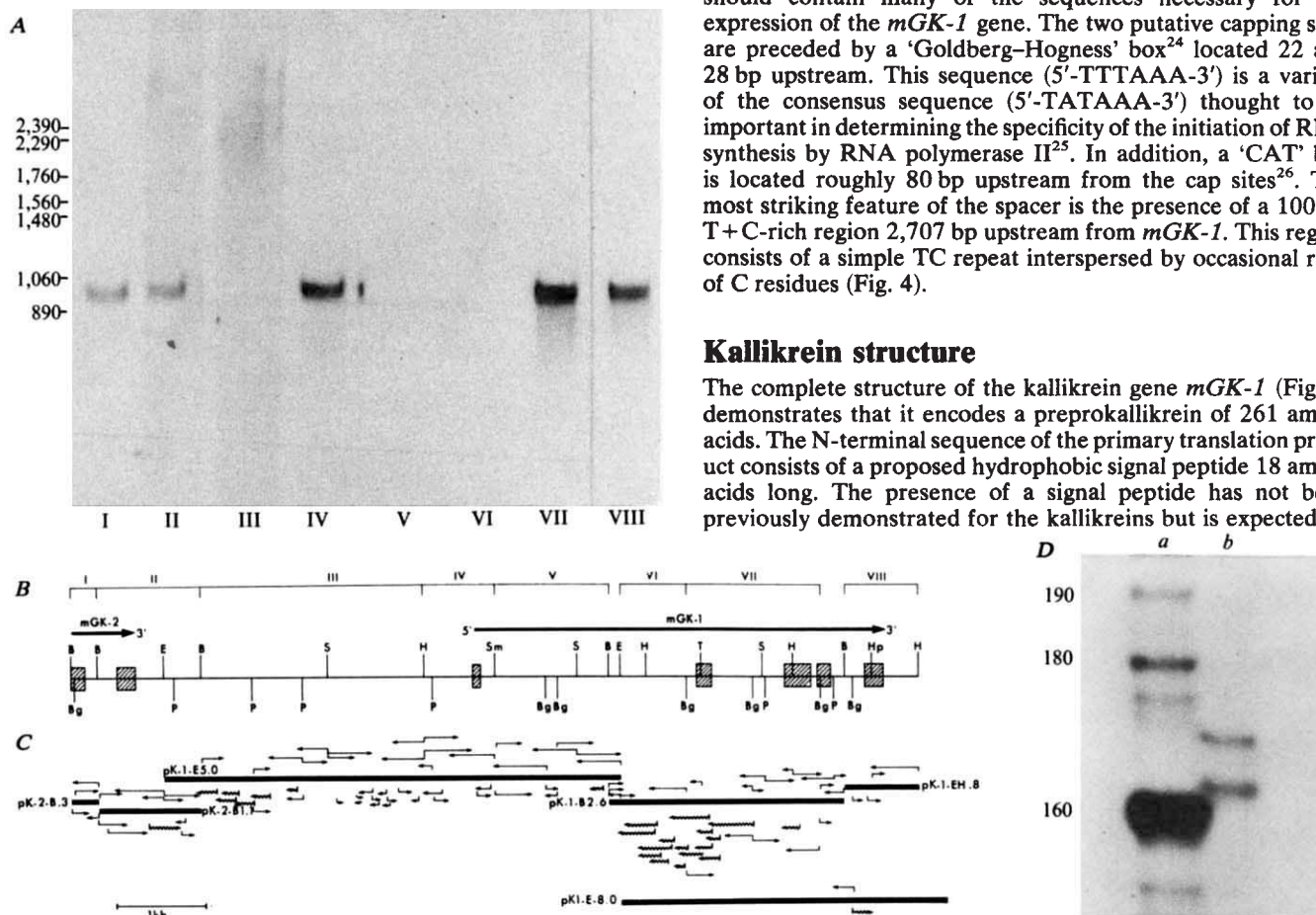
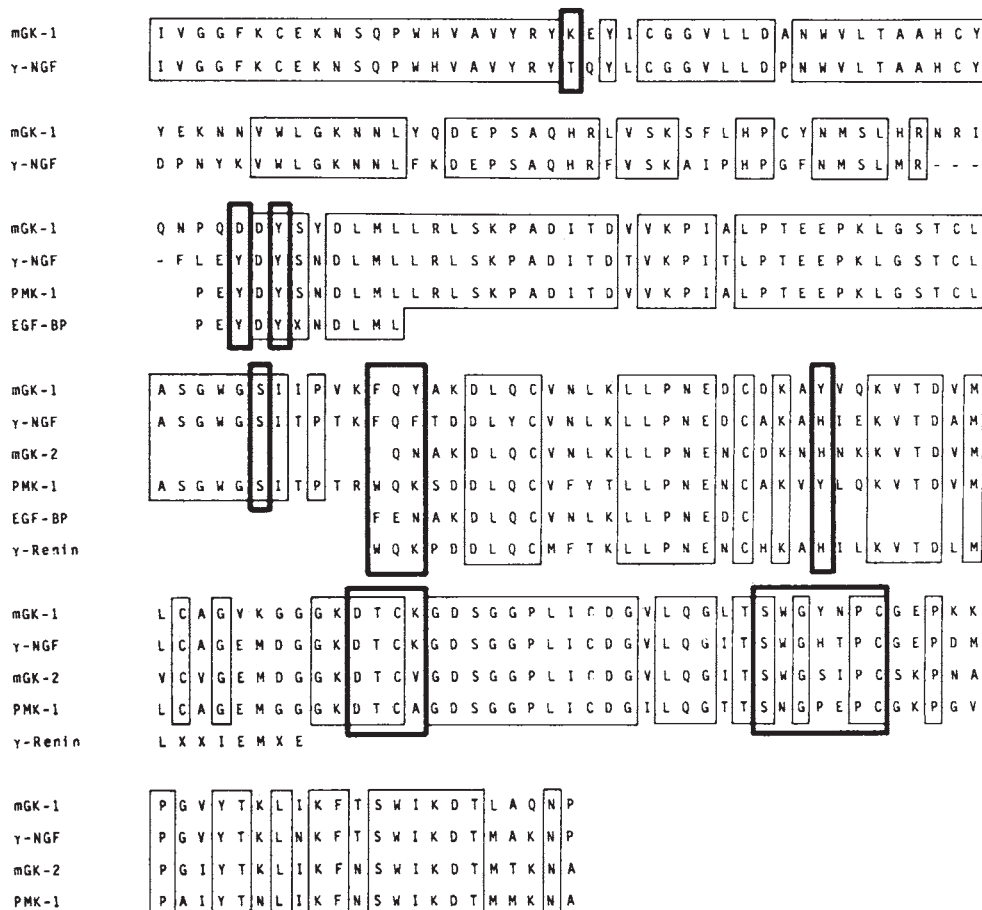


Fig. 3 Map of the sequenced 9.5-kb portion of λ MSP-1. **A**, Northern blot analysis of poly(A)⁺ mRNA isolated from male mouse submaxillary gland. Northern blots were performed essentially as described elsewhere³⁵, using defined restriction fragments (I–VIII) as hybridization probes. Fragments I, II, IV, VII and VIII hybridize to a common mRNA size of ~950 bases. Size markers (as shown) are the eight RNA species from an influenza type A laboratory recombinant H3N2 strain³⁶. RNA blotting was used to approximate the intergenic space and exon–intron boundaries of *mGK-1* and *mGK-2*. **B**, Restriction map of the region containing *mGK-1* and the 3' end of *mGK-2*. Both genes are transcribed in the direction shown by the heavy arrows. Sites are abbreviated as follows: B, *Bam*HI; Bg, *Bgl*III; E, *Eco*RI; P, *Pst*I; S, *Sac*I; Sm, *Sma*I; T, *Taq*I; Hp, *Hpa*II. Only the *Taq*I and *Hpa*II sites used for sequence analysis are shown. The 5' *Bam*HI site within the *mGK-2* gene is the end point of the clone and therefore may only represent an *Mbo*I site in the genome. Boxes denote exons of the respective genes *mGK-1* and *mGK-2*. **C**, Sequencing strategy. The pBR322 subclones used for sequence determination are shown as solid lines. The arrows above and below the lines give the direction and length of sequence obtained. Sequences indicated by arrows above the subclones were obtained by the chemical degradation method³⁷. The arrows below the subclones indicate dideoxy sequencing³⁸ of a defined (*Alu*I, *Sau*3A, *Hae*III or *Rsa*I) restriction fragment subcloned into M13 mp8 or mp9 (ref. 39). Wavy lines indicate sequence data obtained by cloning a series of *Bal*31 exonuclease-generated deletions into mp8. For example, the plasmid pK-1-B2.6 was linearized within the pBR322 sequences by the endonuclease *Cla*I, digested with *Bal*31 and aliquots taken at specific time intervals. By this procedure, it was possible to generate a range of defined deletions from the *Cla*I site towards the *Eco*RI site within the 2.6-kb *Bam*HI insert. In the conditions used the exonuclease removed 400 bp from each end of the linearized plasmid per min (data not shown). These deleted plasmids were then cleaved with *Eco*RI and the fragments subcloned into *Eco*RI–*Hinc*II-cleaved M13 mp8 DNA. Recombinant phage were selected by hybridization to λ MSP-1 DNA and sequenced using a synthetic DNA primer³⁹. The DNA cloned in the plasmids pK-2-B1.1, pK-1-E8.0 and pK-1-E5.0 was also partially sequenced by this method. We have found that this method is more efficient than conventional techniques as it does not depend on the presence of defined restriction sites and the sequence obtained is unidirectional. The sequence of the other strand can easily be obtained by repeating the procedure using a plasmid which has the insert in the opposite orientation. **D**, Initiation of transcription of *mGK-1*. The primer extension analysis was performed essentially as described by Hagenbuchle *et al.*²¹. A 58-bp *Taq*I–*Rsa*I fragment from within exon 2 (nucleotides 6,998–7,056) of *mGK-1* was isolated from pK-1-B2.6 by polyacrylamide gel electrophoresis. After electroelution, the primer was dephosphorylated with calf intestinal alkaline phosphatase (Collaborative Research) and 5'-labelled³⁷ using [γ -³²P]ATP (Amersham) and T4 polynucleotide kinase (Biolabs); 5 pmol of labelled primer were hybridized with 30 μ g of male mouse submaxillary gland poly(A)⁺ mRNA in 50 μ l of 80% formamide, 0.4 M NaCl, 40 mM PIPES pH 6.5, 1 mM EDTA, at 50 °C for 1 h. The reaction was terminated by ethanol precipitation and taken up in 40 μ l of reverse transcriptase buffer containing 1 mM each of the four deoxynucleotide triphosphates. After the addition of 30 units of reverse transcriptase (Life Sciences, Inc.) the reaction mix was incubated at 42 °C for 1 h. The products were ethanol-precipitated and treated with 0.1 M NaOH, before sizing on a 10% sequencing gel (lane b). End-labelled *Hpa*II fragments of pBR322 were used as size markers (lane a).

[illegible]

Fig. 4 Sequence of the mouse *mGK-1* and *mGK-2* gene region. Only the sense strand is shown. The coding sequences which make up the five exons of the complete *mGK-1* gene and the 3' two exons of *mGK-2* have been translated. The arrows in exon 2 of *mGK-1* define the proposed boundaries between the signal peptide, zymogen peptide and the active protease. The CAT box²⁶ and the variant 5'-TTTAAA-3' Goldberg-Hogness box²⁴ preceding *mGK-1* are overlined. The two putative initiation sites of RNA transcription for *mGK-1* are marked by triangles. The polyadenylation site sequences 5'-AATAAA-3'²³ found in the 3' untranslated regions of *mGK-1* and *mGK-2* are boxed. Asterisks indicate the transcription termination sites.

Fig. 5 Comparison of the coding potential of *mGK-1* and *mGK-2* with other mouse kallikrein amino acid sequences. The predicted amino acid sequence of *mGK-1* and *mGK-2* is aligned with the amino acid sequence of γ -NGF⁹ and the partial sequences of EGF-BP⁸, γ -renin (C. D. Bennett and M. Poe, personal communication) and the predicted sequence from the cDNA clone pMK-1 (ref. 14). The boxed areas denote regions of the sequences which have identical residues. The residues believed to line the substrate-binding pocket³⁰⁻³² are heavily boxed. From the alignment of the sequences it seems that four amino acids have been removed from γ -NGF during maturation. The position at which this occurs (indicated by dashes) lies within the autolysis loop where chain breakage is known to occur in the γ -NGF molecule⁹.



the basis of their localization in secretory granules⁷. Analogous to trypsin²⁷, the active kallikrein of 237 amino acids is preceded by a short 6-amino acid zymogen peptide. This prediction of a prokallikrein having a short zymogen sequence is consistent with the isolation of an inactive kallikrein which can be proteolytically activated without significant change in molecular weight³. Kallikreins are known to be glycosylated; the typical glycosylation site of γ -NGF (Asn-Met-Ser)⁹ is also found in the same position in *mGK-1*. The coding potential of *mGK-1* predicts an active serine protease with a histidine, serine and aspartic acid residue in the correct spatial configuration necessary for the formation of a serine protease catalytic site^{28,29}. The presence of aspartic acid at position 183 (Fig. 6) also gives the protease the specificity needed for cleavage at basic residues²⁹. These four amino acid residues are encoded on different exons (see Fig. 6). These data are consistent with the evolution of a serine protease by the formation of an active site through the joining of different exons each encoding an amino acid sequence crucial to the final tertiary structure of the catalytic pocket.

Figure 5 compares the predicted amino acid sequences of *mGK-1*, *mGK-2* and pMK-1 with the known amino acid sequences of γ -NGF and the partial sequences of EGF-BP and γ -renin. As all these proteases share extensive amino acid sequence homologies (73–80%), it is clear that γ -NGF, EGF-BP and γ -renin are encoded by members of the same kallikrein multigene family as the clones isolated here. EGF-BP and γ -NGF are highly homologous but differ in their substrate specificities^{1,2}, a function attributed to alterations in the residues which make up the substrate-binding pocket of serine proteases³⁰⁻³². The residues that are believed to line the substrate-binding pocket³⁰⁻³² are illustrated in Fig. 5. The kallikreins γ -NGF, EGF-BP and γ -renin, as well as those encoded by *mGK-1*, *mGK-2* and pMK-1, show distinctly less homology in these particular residues than in the rest of the sequence. It

is therefore apparent that each of these kallikreins exhibits a different substrate specificity.

Discussion

Kallikrein enzyme activity has been implicated by *in vitro* and *in vivo* studies in a wide variety of essential physiological functions, in addition to its role as a kininogenase. We have found that kallikreins are encoded by a large gene family of 25–30 members having approximately 75% amino acid sequence homology. Each of the sequences so far analysed differs at amino acid residues implicated in the substrate specificity of serine proteases. Thus, it is apparent that functional differences may exist between most, if not all, of the kallikrein gene family members. The diversity of physiological action *in vivo* may, therefore, reflect the composite activities of individual members of the family. Given the known role for three of these enzymes—glandular kallikrein, EGF-BP and γ -NGF—it seems likely that the kallikrein family has a general role in bioactive peptide processing.

The assignment of all kallikrein gene sequences to one

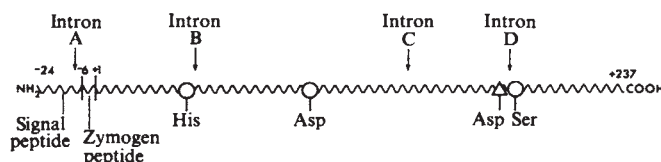


Fig. 6 Position of exon–intron boundaries in the *mGK-1* gene. The position of introns A–D are shown in relation to the structure of the preprokallikrein encoded by *mGK-1*. The three circled residues (His, Asp and Ser) are crucial to the formation of a serine protease catalytic site^{28,29}. The aspartic acid residue represented as a triangle confers on a serine protease the ability to cleave at lysine or arginine basic residues²⁹.

chromosome and their frequent tight linkage suggests that the kallikrein genes are clustered in one locus. The physical linkage of the genes should facilitate a complete structural analysis of the family with a view to understanding the diversity of physiological action. The chemical synthesis of specific oligonucleotide probes based on DNA sequence data obtained from each of the kallikrein genes should allow us to investigate their tissue-specific expression.

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LETTERS TO NATURE

A radio pulsar in the Large Magellanic Cloud

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A high-sensitivity search for pulsars in the Large Magellanic Cloud (LMC) is in progress using the 64-m radio telescope at Parkes, New South Wales. About 7 square degrees have been searched to date, yielding the first extragalactic radio pulsar, PSR0529–66, and several promising but weaker candidates which require further observations. We report here that the new pulsar has a period of 0.9757141 s, a dispersion measure of 125 cm⁻³ pc, and a mean flux density of 1.4 mJy at 645 MHz. Its position is RA = 05 h 29 min 30 ± 1 s, dec = –66°57' ± 8' (1950.0).

The observations were made in December 1980 and May 1981. A dual-mode, linearly polarized feed was used for the telescope, followed by a two-channel FET receiver whose outputs were combined so that the system responded to left-handed circularly polarized waves. A system noise temperature of 65 K was obtained in the direction of the LMC. The output from the receiver was fed to a 256-channel filter bank and a post-detection de-disperser (G. R. A. Ellis and G. A. Gowland, in preparation). The de-disperser provided eight simultaneous outputs corresponding to different dispersion measures; the search was conducted with these set for dispersion measures of 7, 106, 205, up to 700 cm⁻³ pc. The system had a total bandwidth of 25.6 MHz. Each beam position was observed for 2 h. In the December 1980 session, each dispersion channel was sampled 25 times per second, whereas in May 1981 a sample rate of 200 Hz was used. In each case the raw data were recorded on magnetic tape for subsequent processing. Throughout the May 1981 observing session, weak pulsed noise signals were injected into the system to monitor the system

sensitivity. A variety of calibration pulse periods, amplitudes and widths were used for this purpose.

The off-line processing which was applied to all eight dispersion channels consisted of searching the Fourier transform of the data for candidates and selecting the best five. The original data were then folded for a range of periods centred on the candidate periods and the signal-to-noise ratio determined. Tests using the pulsed calibration signal showed that, while the sensitivity of the system depended on the sky temperature, in most cases a pulsar with a mean flux density of 1.2 mJy would have been selected as a candidate for folding. A pulsar of this strength results in a signal-to-noise ratio of 25:1 in the folded data and although a signal-to-noise ratio considerably less than this would be significant, choosing the correct candidate periods for folding is a major problem. The quantity of data precludes folding for more than about five candidates for each data stream. The selection criteria that we have used successfully identify the pulse calibration signals for folding when they exceed 1 mJy mean, but as the signal strength is reduced from this the selection process becomes less reliable.

We have searched 29 beam positions in the LMC with this system (beamwidth 25 arc min), and have detected one pulsed source with a mean flux exceeding 1.2 mJy. Several beam positions show candidates with signal-to-noise ratios of 8–10, but further observations of these are needed.

The pulsar was found during the May 1981 session in the beam position centred on (RA 04 h 30 min, dec –67°00'). The appropriate data from the December 1980 session were folded at the same period and the pulsar was again detected. Further observations in February 1982 gave an improved position of RA = 05 h 29 min 30 s ± 1 min, dec = –66°57' ± 8' (1950.0). It was detected in the three dispersion channels corresponding to 7, 106 and 205 cm⁻³ pc, with the narrowest profile in the 106 cm⁻³ pc channel. Using the profile widths in the three channels, we estimate that the dispersion measure is 125 cm⁻³ pc. The period of the pulse is 0.97571407 ± 3 s at epoch JD 2444732.7.

Figure 1 shows the integrated pulse profile from the 2-h observation in May 1981 using the 106 cm⁻³ pc dispersion channel of the de-disperser. The resolution is 100 samples per period. The pulse width at this dispersion is ~4% of the period.

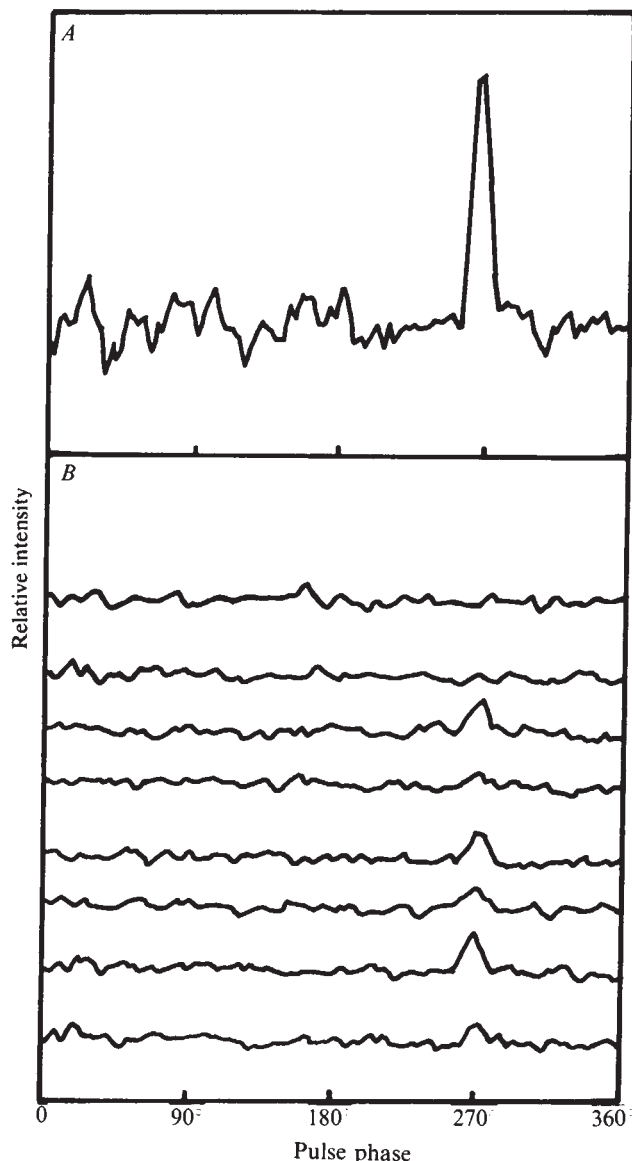


Fig. 1 A, The integrated pulse profile of PSR0529-66 from a 2-h observation at a dispersion measure of $106 \text{ cm}^{-3} \text{ pc}$. B, The data from A summed in eight consecutive 15-min integrations.

Figure 1 also shows the profiles obtained when the data are grouped into eight successive 15-min integrations. The pulse is clearly visible in most of the integrations, but there is much variability from one to another, indicating that the pulse amplitude fluctuates on a time scale of tens of minutes over the 25-MHz system bandwidth.

The association of this pulsar with the LMC is inferred from its direction and dispersion measure (DM). The mean free electron density in the local neighbourhood is $\sim 0.025 \text{ cm}^{-3}$. If we assume that this density does not decrease with distance (z) from the galactic plane, this pulsar must be 5 kpc distant and more than 2.5 kpc from the galactic plane. Its z -component of DM, given by $\text{DM} \sin |b|$, is $68 \text{ cm}^{-3} \text{ pc}$. This can be compared in Fig. 2 with the distribution of $\text{DM} \sin |b|$ for 330 pulsars¹. The distribution is consistent with a model in which the electron density falls with z as e^{-z/z_0} , where $z_0 \sim 1 \text{ kpc}$ (ref. 2). In this model, the maximum galactic contribution to the z -component can only be $\sim 25 \text{ cm}^{-3} \text{ pc}$, unless the line of sight passes through a galactic H II region. Several of the pulsars with $20 \leq \text{DM} \sin |b| \leq 40 \text{ cm}^{-3} \text{ pc}$ are known to be so affected. No H II regions near the direction of this pulsar have been reported except those known to lie in the LMC. Furthermore, O-type and very early B-type foreground stars that could excite dense compact H II regions are very rare in the direction of the LMC³. A

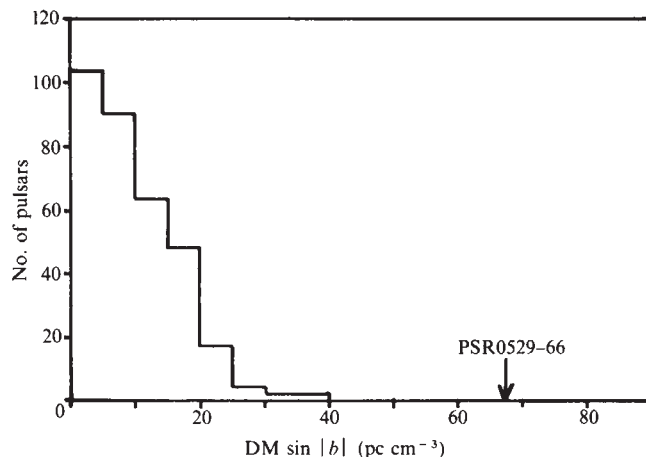


Fig. 2 The distribution of $\text{DM} \sin |b|$ for 330 galactic pulsars. The position of PSR0529-66 is shown by the arrow.

search of survey plates from the UK Schmidt Telescope and available star catalogues in the region near the pulsar shows no foreground O or B star within 2° of this position. We also looked for dense obscuration that could hide foreground H II regions; none were seen though any such obscuration would have been obvious from its effect on light from the diffuse emission nebulae that pervade this region of the LMC. However, the region of the LMC in the direction of the pulsar is rich in young Population I objects; pulsars are believed to be young Population I objects. We, therefore, conclude that the pulsar is in the LMC.

The pulsar has a mean flux of 1.4 mJy with a peak flux of $\sim 30 \text{ mJy}$, and at the 50 kpc distance of the LMC it has a luminosity of $3,500 \text{ mJy kpc}^2$. The observed range of luminosities of galactic pulsars is 10^{-1} – 10^5 mJy kpc^2 . The properties of PSR0529-66 are therefore similar to those of pulsars in our own galaxy.

Long *et al.*⁴ have published a soft X-ray survey of the LMC in which they listed 97 sources. Their source no. 44 at RA 05 h 00 min 06 s, dec $-66^\circ 56' 13''$, is within 8 arc min of our best position. They identify this source as a compact variable with a very hard spectrum. A more accurate position for PSR0529-66 is required to check this association.

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Oblateness effects on the spindown of fast pulsars

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The recent discovery^{1,2} of the very rapidly rotating pulsar PSR1937+214 has triggered off many theoretical speculations^{3–5}. Assuming that the pulsar is a fast-rotating superdense fluid, we show here that the increase in the moment of inertia and in the gravitational self-energy of the spheroid due to its oblateness lead to substantial differences in the time evolution of the pulsar from that predicted by the standard models. Careful observations of its $\dot{P}$, $\ddot{P}$ and its spectrum can put constraints on the properties of neutron star matter. Even with the existing observation of $P = 1.56 \text{ ms}$, considerations of dynamical stability of the rapidly rotating neutron star yield a lower limit $\bar{\rho} > 1.7 \times 10^{14} \text{ g cm}^{-3}$ for the mean stellar density.

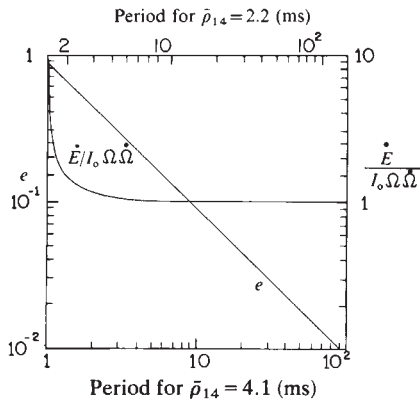


Fig. 1 Rate of change of the total energy of a rotating fluid neutron star in units of the rate expected for a rigid body. The eccentricity of the spheroid plotted against the pulsar period is also shown

Spheroids are among the shapes taken at equilibrium by rotating, self-gravitating bodies⁶. These become the MacLaurin spheroids when the elastic stress energy is negligible compared with the rotational and gravitational energies (as is the case for typical stress energies stored in neutron star crusts⁷). The crucial new feature of our work is the inclusion of the oblateness effects in the moment of inertia I

$$I = \frac{2}{5} MR^2(1 - e^2)^{-1/3} = I_0(1 - e^2)^{-1/3} \quad (1)$$

and in the gravitational self-energy E_G (ref. 8)

$$E_G = -\frac{3}{5} \frac{GM^2}{R} (1 - e^2)^{1/6} \frac{\sin^{-1} e}{e} \quad (2)$$

of the spheroidal pulsar, whose eccentricity e is given by⁶

$$\frac{\Omega^2}{\pi G \bar{\rho}} = 2e^{-3}(3 - 2e^2)(1 - e^2)^{1/2} \sin^{-1} e - 6e^{-2}(1 - e^2) \quad (3)$$

where G is the gravitational constant; Ω is the angular speed; M the mass; and r the radius of the pulsar. Clearly equation (3) is exactly valid only for a star with a uniform density throughout and will be used here to indicate the effects of the changing oblateness on the evolution of the pulsar. Calculations simultaneously taking into account the density stratification for rapidly-rotating neutron stars along with the effects of general relativity are not yet available.

The spindown of such a pulsar due to the emission of electromagnetic radiation by a frozen-in magnetic dipole is described by

$$\dot{E} = \frac{d}{dt} \left(\frac{1}{2} I \Omega^2 + E_G \right) = -\frac{2}{3} \frac{\mu^2}{c^3} \Omega^4 \quad (4)$$

which clearly differs from the standard form⁹

$$I_0 \Omega \dot{\Omega} = -\frac{2}{3} \frac{\mu^2}{c^3} \Omega^4 \quad (5)$$

Here μ is the transverse component of the magnetic moment. Consistent with the assumption of axisymmetry, we neglect the emission of gravitational radiation, as our main purpose is to illustrate the effects of rotational deformations.

Our numerical solutions of equations (1)–(5) are shown in Figs 1 and 2. We have made the following assumptions about the properties of the neutron star:

- (1) $M = 1.4 M_\odot$.
- (2) $1.7 \times 10^{14} \text{ g cm}^{-3} < \bar{\rho} < 4.1 \times 10^{14} \text{ g cm}^{-3}$. The upper limit corresponds to a neutron star obeying the Friedman–Pandharipande¹⁰ equation of state, and the lower limit follows from the condition that the new ($P = 1.56 \text{ ms}$) fast pulsar should not be dynamically unstable⁶ ($\Omega^2/\pi G \bar{\rho} < 0.45$).
- (3) $\mu \approx 10^{27} \text{ G cm}^3$. This is obtained from the estimated luminosity of the new pulsar¹² which is $\sim 10^{36} \text{ erg s}^{-1}$, while the

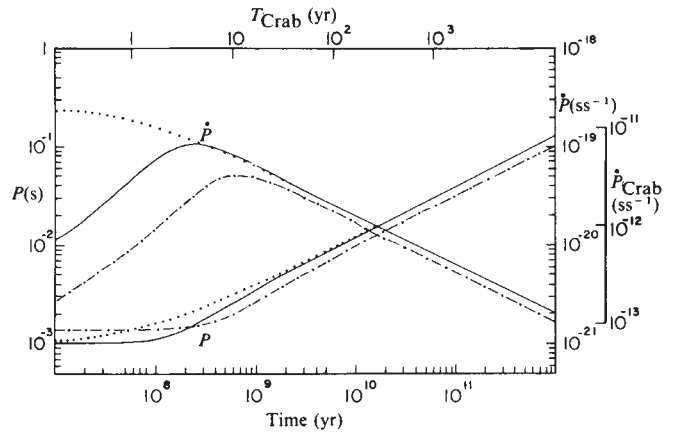


Fig. 2 Evolutionary tracks for the new pulsar born near the limit of dynamical stability with average density $\bar{\rho}_{14} = 4.1$ (solid lines) and with $\bar{\rho}_{14} = 2.2$ (— — — lines), including the effects of rotation-induced oblateness along with that for the standard rigid rotator model with $\bar{\rho}_{14} = 4.1$, for comparison (dotted lines). The same tracks also represent the evolution of the Crab pulsar when read against the scaled t and $\dot{P}$ axes.

pulsar in Crab with a surface field¹¹ of about $3 \times 10^{12} \text{ G}$ has a luminosity $\sim 10^{38} \text{ erg s}^{-1}$, and takes into account the intensity of magnetic dipole radiation which scales as $\mu^4 \Omega^2$ [see equation (4)]. (Alternatively, if we compare their radio luminosities² and assume that these are the same fraction of their total luminosities, as the pulse characteristics are so similar¹², then $\mu \approx 10^{28} \text{ G cm}^3$. With this larger magnetic moment the observed $\dot{P} \sim 10^{-19} \text{ s s}^{-1}$ would imply $\bar{\rho} = 1.72 \times 10^{14} \text{ g cm}^{-3}$ and an age of $\sim 10^5 \text{ yr}$).

Figure 1 shows that the increase of $\dot{E}$ over the value $I_0 \Omega \dot{\Omega}$ (given by the standard model) becomes substantial at short periods, where the rotational oblateness is considerable. The eccentricity of the spheroid is also shown. Figure 2 shows the effects of high eccentricities on the time evolution of P and $\dot{P}$, because these bear directly on the new pulsar. With oblateness effects included, $\dot{P}$ no longer increases monotonically with decreasing P (unlike in the standard model, the results of which are shown by dotted lines); rather, it goes through a maximum and drops rapidly for very short periods. Whereas $\dot{P} < 0$ for slow pulsars (as in the standard model), $\dot{P} > 0$ for very fast pulsars, the latter being a characteristic signature of, and therefore an observational test for, the oblateness effects. Further, given an independent estimate of the magnetic moment, measurements of P and $\dot{P}$ determine the location of the pulsar within the allowed range of neutron star densities (see Fig. 2) and can constrain equations of state for dense matter. The central value of the recent¹² result $\dot{P} = (0.4 \pm 0.7) \times 10^{-19} \text{ s s}^{-1}$ corresponds to $\bar{\rho} = 2.2 \times 10^{14} \text{ g cm}^{-3}$; however, the uncertainties are still large enough to allow almost the entire range of densities from $\bar{\rho}_{\min} = 1.7 \times 10^{14} \text{ g cm}^{-3}$ derived from considerations of dynamical stability up to $\bar{\rho}_{\max} = 4.1 \times 10^{14} \text{ g cm}^{-3}$ implied by the hardest equation of state¹⁰.

Figure 2 also gives the 'age' of a fast pulsar (variously definable as the time taken in spinning down from any one of the following critical points: (1) dynamical instability point, where $\Omega^2/\pi G \bar{\rho} = 0.45$, $e = 0.95$; (2) secular instability point and bifurcation point for MacLaurin–Jacobi Sequences, where $\Omega^2/\pi G \bar{\rho} = 0.37$, $e = 0.81$). This age increases by a factor of 3–10 over the age calculated without taking rotational oblateness into account; the estimated age of the new pulsar is $2\text{--}3 \times 10^8 \text{ yr}$.

Indeed, our calculation shows that the effects of rotational oblateness are bound to have an important role for all fast pulsars, irrespective of the conditions prevailing at their births. Also, because the oblateness causes a substantial reduction in $\dot{P}$ at small values of P one would expect to find a corresponding increase in the population of fast pulsars with periods shorter

than a few milliseconds. Concerted searches for fast pulsars in the next few years should be able to test these predictions.

Finally, since the intensity of gravitational radiation increases as Ω^6 , significant emission of such radiation from the new pulsar may be expected even if the departure from axisymmetry is as small as 10^{-6} in the equatorial plane of the pulsar. The possibility that these gravitational waves may be directly observed using modern techniques clearly exists. We will consider elsewhere gravitational radiation effects, and also the effect of the torques that tend to align the rotational and magnetic axes, and that of stress energy. We emphasize here that the changes in the moments of inertia and in the gravitational self-energies have a crucial role in the evolution of fast pulsars, and that a positive $\dot{P}$ and a relatively high concentration of rapidly rotating pulsars are signatures of this effect.

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Tentative confirmation of an aurora on Uranus

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There have been three recent reports of the detection of Ly α radiation (due to atomic hydrogen, H, at wavelength 1,216 Å) from Uranus by means of the International Ultraviolet Explorer (IUE) satellite. The interpretations of these results differ. Two reports conclude that there is a strong aurora on Uranus, but the third concludes that the source is resonance scattering of solar Ly α . We report here detection of emission features due to molecular hydrogen, H₂, near 1,600 Å. This detection is near the limit of the IUE sensitivity. If it is real, the detection of H₂ emission strongly supports the conclusion that Uranus has an aurora comparable in strength with those of the inner two giant planets, Jupiter and Saturn. We also present the first published IUE spectra of the Saturn aurora.

Durrance and Moos¹ reported their discovery of intense Ly α radiation, amounting to a brightness of 1.6 ± 0.4 kR, which they attributed to a uranian aurora, caused by energetic particles in the uranian magnetosphere which dissociate molecular hydrogen (H₂) and subsequently excite atomic hydrogen (H). Clarke² independently announced the discovery of a uranian aurora, based on his additional observation of variability of Ly α from 0.43 to 0.86 kR over a 2-day interval.

Fricke and Darius³ reported Ly α brightnesses ranging from 0.2 to 1.2 kR over several years. Although their observations were similar to those of Durrance and Moos, and of Clarke, their interpretation was that their Ly α signal was most likely due to resonance scattering of solar Ly α by H.

The hypothesis of Fricke and Darius would not be tenable for Jupiter and Saturn, where aurorae have been unambiguously observed by a variety of spacecraft. However, physical conditions on Uranus are so different from the inner giant planets that consideration of different radiative mechanisms is appropriate. In particular, the atmosphere of Uranus is sufficiently cold that the abundance of methane (CH₄) should be greatly reduced in its stratosphere due to condensation at lower altitudes⁴. Since CH₄ is a strong absorber at the Ly α wavelength, its absence from the stratosphere of Uranus would greatly increase that planet's ability to scatter solar Ly α back to the observer without absorption, contrary to the situation on Jupiter and Saturn. Thus, within the limitations of their monochromatic observation, the conclusions of Fricke and Darius are qualitatively reasonable.

Observations of H₂ emission features at 1,575 and 1,608 Å are useful for resolving the different claims for two reasons. First, CH₄ does not absorb longward of 1,500 Å, and so it does not influence the 1,600-Å H₂ features. And second, there are no solar features at those wavelengths to be resonantly scattered. The only plausible means of exciting these bands is through collisions between H₂ and precipitating magnetospheric particles.

We have previously obtained long-duration spectra of Uranus and other objects⁵ with the short-wavelength camera (SWP) on board the IUE. Our original objective was to study the spectral region of reflected sunlight, which is longward of $\sim 1,650$ Å, to search for possible absorption features of trace molecules. When the discoveries of the Ly α radiation were announced, we examined all our data, including some that are previously unpublished, at wavelengths below 1,650 Å to see if there was also an auroral emission due to H₂.

We selected four exposures for study (see Table 1) all taken with the small aperture of the SWP, having exposure times ranging from 5 to 14 h. To accomplish the 14 h exposure, it was necessary to begin when the spacecraft was under the control of the European ground station at Madrid and to terminate from the US ground station at Greenbelt. Exposure times longer than about 14 h are precluded by background noise from the Earth's natural radiation belts.

The small aperture was chosen in the belief that it would be better for guiding on the planet. Its nominal diameter is 3 arc s, so that Uranus slightly overfills it. It is possible to guide on the spill-over light. However, this aperture has two disadvantages. One is that the amount of spill-over light is variable and irreproducible, so that the absolute calibration is uncertain by up to a factor of 2. Fortunately, there is no evidence that this defect produces relative errors within a spectrum. Only the scale is affected. The other disadvantage is that there is an instrumental blemish, a reseau mark, that is required for constructing the spectral image and is unfavourably placed with respect to H₂ emission.

The results of summing the four Uranus spectra are shown in Fig. 1, which also includes the solar spectrum of Mount and Rottman⁶. Toward the right of Fig. 1, reflected sunlight, which decreases with decreasing wavelength, is apparent. The exposures are all sufficiently long to be saturated at Ly α by the geocorona, producing no useful information there. The unfortunate reseau mark is indicated by a small gap in the data points near 1,590 Å.

The error bars in Fig. 1 were determined as follows: The camera background spectra are extracted on either side of the individual spectra. The mean standard deviation in a 15 point data window is calculated for every point in the background data and assigned to the corresponding point in the spectrum. The four individual spectra have been combined by computing the weighted mean at every data point. The resulting combined spectrum has been smoothed with a 11-Å wide gaussian filter. When combined with the 7-Å width of the small aperture, this gives a 13-Å spectral resolution, which qualitatively matches laboratory results presented below in Fig. 2. The 1σ error of the spectrum shown in Fig. 1 is obtained by

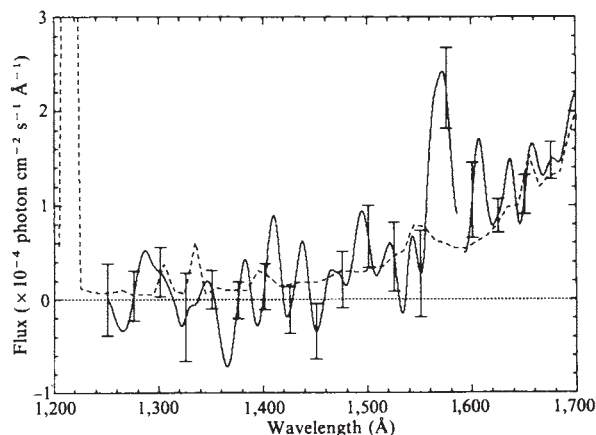


Fig. 1 Average of four SWP spectra of Uranus (solid line) smoothed with an 11 Å wide gaussian filter which produces a 13-Å resolution because of the 7 Å width of the aperture. The gap near 1,590 Å indicates a reseau mark. The error bars are discussed in the text. The solar spectrum of Mount and Rottman⁶, normalized to our data at 1,700 Å, is included for comparison (dashed line).

standard propagation of the errors of the four individual spectra.

There is a very prominent emission feature near 1,570 Å and another less prominent one near 1,610 Å. It is on the basis of these features, which are close to the well known H₂ features at 1,575 and 1,608 Å, that we suggest that there is an H₂ aurora on Uranus as well as the H aurora. Below 1,600 Å, H₂ has other spectral features, but none are bright enough to be reliably identified individually.

The features near 1,600 Å are visually apparent in the raw data of all four of our exposures, and they are enhanced by the averaging process. Other 'features' such as those near 1,400 Å in Fig. 1 are not present in all spectra, and are greatly reduced by averaging.

Figure 2 shows a spectrum of Saturn for comparison. These data were taken with the large aperture, which is a 10 by 20 arcs ellipse with the major axis aligned approximately north to south. The aperture therefore contains both northern and southern polar limbs, but excludes the equatorial limbs of Saturn. The signature of the rings is very small in Fig. 2. Coincidentally, the respective planets overfill the apertures used to observe them by similar amounts, and each spectrum includes light from polar regions. The Saturn spectrum in Fig. 2 is, therefore, suitable for direct comparison with the Uranus spectrum of Fig. 2.

Also shown in Fig. 2 is a laboratory spectrum of an H₂ discharge lamp, obtained by Durrance *et al.*⁷. The Saturn H₂ auroral features at 1,575 and 1,608 Å, superimposed on the decreasing reflected light component, are clearly visible. There are also features on Saturn below 1,300 Å that are consistent with H₂ emission. The absolute scale for the Saturn spectra,

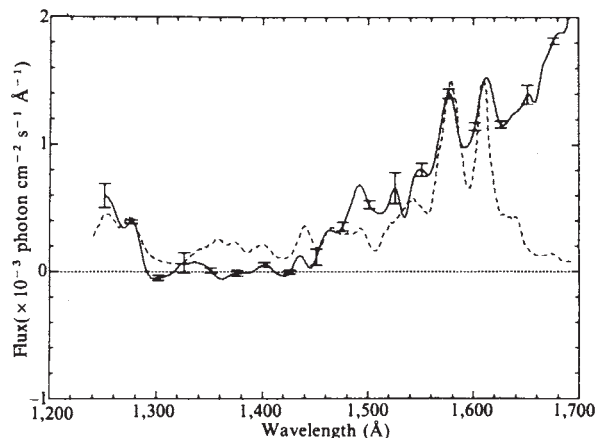


Fig. 2 Saturn spectrum (solid line) compared with the laboratory H₂ emission spectrum of Durrance *et al.*⁷ (dashed line). Note the offset zero point.

which were taken with the large aperture, is much more reliable than that of Fig. 1.

In both the Saturn and laboratory spectra, the two H₂ peaks have comparable height. The peaks are also equal on Uranus, within the noise levels. It is possible that the reseau has distorted adjacent spectral regions, but its nominal width seems to be much less than the space between the peaks. The large aperture Saturn spectra were recorded on a different part of the camera faceplate and so do not have the same reseau contamination.

One encouraging feature about the tentative identification in Fig. 1 is that the brightness of the uranian aurora is very nearly the same as the saturnian one in Fig. 2. The Saturn H₂ peaks are about 6×10^{-4} photons $\text{cm}^{-2} \text{s}^{-1} \text{Å}^{-1}$ above their background level and the mean value of the uranian peaks is 2×10^{-4} photons $\text{cm}^{-2} \text{s}^{-1} \text{Å}^{-1}$. In both cases, almost all the light from the planet including aurorae enters the respective apertures, so that if Uranus were as close as Saturn, its signal would be roughly four times larger, in very close agreement with the Saturn signal. Thus, the claim of a uranian aurora reported here does not imply the existence of mechanisms not already known elsewhere.

This close agreement should not be overinterpreted. First, there are significant calibration uncertainties in small aperture spectra, as discussed above. Second, since there is no spatial resolution for Uranus, the size of the uranian auroral zone is unknown and its intrinsic brightness cannot be calculated. Third, the two planets differ in many respects, including: mean density and internal structure; obliquity (that is, tilt of the rotational axis); distance from the Sun; and density and distribution of ring particles. These differences could affect respectively: the planetary magnetic field; magnetospheric interaction with the solar wind; strength of the solar wind at the magnetopause; and trajectories of magnetospheric and auroral particles. The agreement between the unresolved auroral fluxes could therefore be coincidental.

Table 1 Uranus small aperture SWP spectra

Image-no.	Date	Exposure time (hours)	Scattering* correction (%)	Normalization† factor	Observation station
SWP 9478	9 July 1980	14	6.7	1	ESA/NASA
SWP 17425	15 July 1982	6.6	9.1	1.42	ESA
SWP 8765	16 April 1980	6.5	8.1	1.12	NASA
SWP 7680	17 January 1980	5	7.6	0.98	NASA

* SWP images of solar type spectra are contaminated by scattered light along the dispersion direction. The spectra have been corrected for that by subtracting a wavelength independent scattered light level determined by the average flux between 1,300 and 1,330 Å. This correction is given here in percentage of the average flux between 1,800 and 1,840 Å.

† Before the individual spectra are averaged together they are normalized to the same mean flux. The normalization factors indicate the relative photometric accuracy of the IUE SWP camera.

It is desirable to repeat the observations of Uranus using the large aperture of the SWP, to improve the quality of the data. We have proposed to NASA to do this. Pending the availability of such data we can claim only a tentative confirmation of the reported aurora on Uranus.

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***In situ* aircraft measurements of enhanced levels of N₂O associated with thunderstorm lightning**

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During the past few years, considerable research activity has concerned atmospheric lightning as a source of the oxides of nitrogen, nitric oxide (NO) and nitrous oxide (N₂O)^{1–10}. Most of this research has centred on NO and has established lightning as a major natural source of this important tropospheric species^{1–6,8–10}. This research has been based mainly on theoretical calculations^{2,4–6,8,9}, laboratory measurements^{3,10}, and on a single measurement of enhanced levels of nitrogen dioxide (NO₂), believed to have resulted from NO produced in thunderstorm lightning¹. We report here the first series of measurements of enhanced levels of atmospheric N₂O associated with thunderstorm lightning. N₂O is an environmentally significant species since its reaction with excited oxygen (O(¹D)) in the stratosphere produces NO which through the catalytic NO_x cycle is responsible for about 65% of the total global destruction of ozone (O₃) in the stratosphere¹¹. In addition, due to its absorption at 7.8 μm in the atmospheric window, N₂O absorbs and then re-emits Earth-emitted IR radiation which leads to an enhancement of the surface temperature, and, hence, has important implications for climate considerations¹².

The present data were obtained from 1980 to 1982 as part of the NASA Langley Research Center Storm Hazards Project. As part of this project an atmospheric sampling system, the Atmospheric Chemistry Experiment (ACE), flew aboard a specially instrumented F-106B Delta Dart aeroplane. The objectives of the Storm Hazards Project are to improve our understanding of the effects of thunderstorm hazards on the design and operation of aircraft and to improve the state of the art of predicting, detection and avoidance of severe storms^{13,14}. The F-106B Delta Dart carries instrumentation to measure the electrical and meteorological environment of the thunderstorm, including the electric and magnetic properties of lightning, turbulence, windshear, and precipitation rates for both rain and hail^{13,14}. Flights into active thunderstorms in the vicinity of both the NASA Langley Research Center in Hampton, Virginia, and NOAA National Severe Storms Laboratory in Norman, Oklahoma, began in 1980 and continued through the 1982 thunderstorm season. During this 3-yr period, the aeroplane made 419 penetrations into thunderstorms, received 176 direct lightning strikes, and obtained 296 samples of air from electri-

cally active thunderstorms. In addition, several hundred samples of fair weather (non-storm) or 'clear air' were obtained during check-out and non-storm flights. These samples were used for determination of the N₂O concentration in clear air.

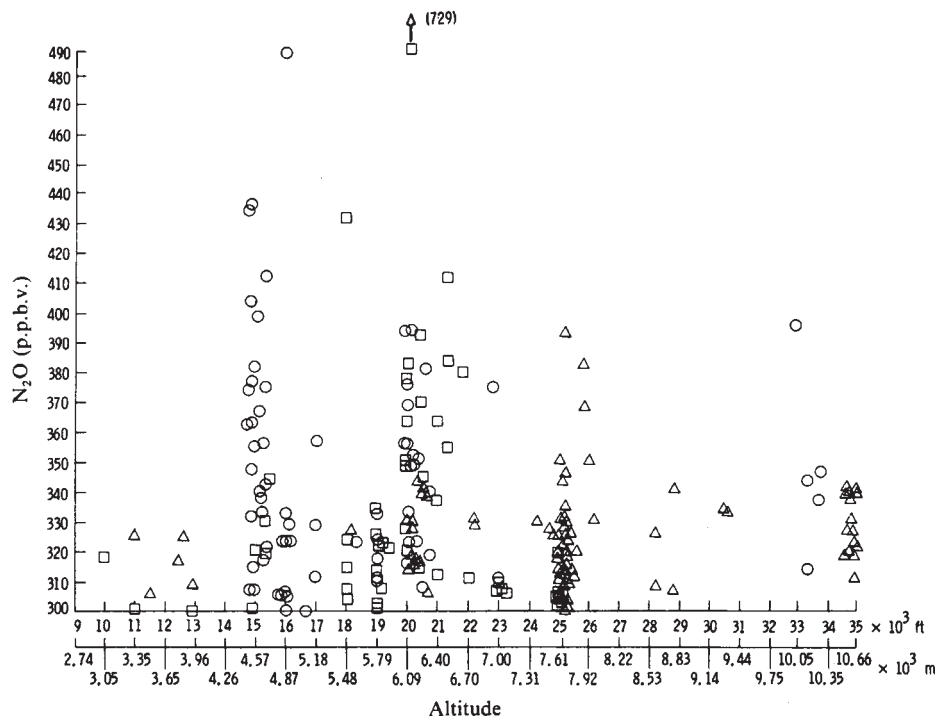
The heart of the ACE sampling system is 24 standard 500-cm³ stainless steel gas sampling bottles. A pitot tube mounted on the right weapons bay door (forward and below the right engine intake) draws in samples of air. The pitot tube is connected to an air pump and the sampling bottles in the aeroplane weapons bay through a 3-foot Teflon tube. The evacuated sampling bottles are filled on command of the flight observer in the aft cockpit who serves as the instrument scientist during the flights. The sampling system fills each bottle to slightly positive pressure and then seals the bottle. The sampling time is set at 10 s. The bottle pressure varies depending on the flight altitude which varies from 11,000 (3,350 m) to 40,000 feet (12,192 m). Most storm penetrations are made at an indicated air speed of about 300 knots. On landing, the sampling bottles are removed from the aeroplane and brought to the gas analysis laboratory. The air sample is analysed for its N₂O content using an 810 F and M gas chromatograph equipped with a Tracor electron capture detector and linearizer with an 8-foot by 1/8 inch o.d. column of Porapak Q held at a temperature of 40 °C (ref. 7). The ⁶³Ni detector is operated at a temperature of 350 °C with a standing current of 1.5 × 10⁻⁷ A. The N₂O calibration was aided by N₂O samples provided by the National Bureau of Standards (NBS) as part of the NBS interlaboratory N₂O calibration network⁷.

For the 1980 and 1982 samples the clear air concentration of N₂O was found to be 308 ± 20 p.p.b.v. (parts per 10⁹ by volume) while for the 1981 samples the clear air concentration of N₂O was found to be 311 ± 20 p.p.b.v. In the analyses of the storm samples, an enhanced N₂O level was arbitrarily defined as a concentration greater than the clear air value by at least 10% (the N₂O measurement standard deviation is about 6.5%). Using this criterion, 78 out of 296 storm samples, or 26% exhibited enhanced concentrations. Maximum N₂O concentrations of 490, 729 and 393 p.p.b.v. were obtained in 1980, 1981 and 1982, respectively. The year-by-year summary of the N₂O measurements is given in Table 1. During the 10-s sampling time, the aeroplane travels approximately 4,000 feet (1,200 m). Hence, the measured concentration of N₂O represents a distance-averaged value which tends to dilute the N₂O concentration in the immediate vicinity of a lightning strike. Multiple penetrations into the same thunderstorm separated by several minutes time indicate that the distribution of N₂O within the thunderstorm is very non-homogeneous. The non-homogeneity of N₂O may be related to the strong vertical motions redistributing N₂O within the storm. Analysis of the storm samples indicates that both the measured concentration of N₂O, as well as the measurement range of scatter, exhibit a slight altitude dependence. The concentration of N₂O from all flights from 1980 to 1982 is plotted as a function of altitude in Fig. 1. Note that the bulk of the measurements were obtained

Table 1 Measurements of N₂O in thunderstorm lightning

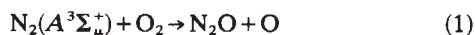
	1980	1981	1982	Totals
No. of storm flights	16	15	12	43
No. of usable storm samples	111	95	90	296
Mean N ₂ O concentration from clear air flights (p.p.b.)	308	311	308	
No. of storm samples with N ₂ O > 10% above clear air value (%)	39 (35%)	25 (26%)	14 (16%)	78 (26%)
Distribution of storm samples				
% Enhancement: 10–19%	23	15	12	50
20–29%	10	6	2	18
30–39%	2	3	—	5
40–49%	3	—	—	3
>50%	1	1	—	2
Maximum storm sample N ₂ O concentration (p.p.b.)	490	729	393	

Fig. 1 The variation of N_2O with altitude based on analyses of air samples obtained in electrically active thunderstorms by direct aeroplane penetrations from 1980 (○); 1981 (□); 1982 (△).



at three altitudes—15,000 (4,570 m) 20,000 (6,090 m) and 25,000 (7,610 m) feet. Also note that maximum N_2O concentrations, as well as maximum measurement scatter occurred at 15,000 feet, with both N_2O concentration and measurement scatter less at 20,000 feet, and even less at 25,000 feet. Field measurements and laboratory tests over the past 6 years indicate that N_2O is very stable, that is, it is not produced or destroyed in the sampling bottle. Because N_2O is not very water soluble, very little N_2O is lost from the thunderstorm environment by precipitation. Due to the inhomogeneity of the measured N_2O within the thunderstorm, as well as uncertainties in the total energy deposited by lightning on a global scale, it is difficult to use these measurements to assess accurately the contribution of lightning to the global budget of N_2O . However, the measurements of enhanced levels of N_2O associated with electrically active thunderstorms reported here represent the first direct and unambiguous measurements of the production of a trace gas by lightning and qualitatively confirm earlier laboratory experiments and theoretical calculations⁷. However, an estimate of the global production of N_2O by lightning can be made by multiplying the experimentally determined yield of N_2O (4×10^{12} molecules J^{-1})⁷ by the total global energy dissipated by lightning (estimated to be about 10^{-8} J cm^{-2} s^{-1})¹⁰. This estimate gives about 300 tons(N) yr^{-1} of N_2O due to lightning.

While biogenic activity is probably the overwhelming source of N_2O in the atmosphere¹⁵, several atmospheric processes that may lead to the production of N_2O have recently been suggested^{16–20}. It has been suggested that the reaction of metastable molecular nitrogen ($N_2(A^3\Sigma_u^+)$) with molecular oxygen (O_2) leads to the production of N_2O by the reaction¹⁶:



This reaction forms N_2O with a quantum yield of $0.6 \pm 30\%$. Metastable N_2 is produced by the impact of low-energy secondary electrons on N_2 and by the absorption of cosmic rays and solar X rays¹⁶. An additional source for the production of metastable N_2 within electrically active thunderstorms may be bremsstrahlung X rays emitted from energetic electrons produced by the lightning discharge. Significant quantities of 3 to >12 keV X rays produced by lightning within the thunderstorm have been measured by an X-ray detector on the F-106B aeroplane²¹. It has also been suggested that the reaction of excited ozone (O_3^*) with N_2 may lead to the production of N_2O

through the reaction¹⁸:



The source of O_3^* may be the three-body recombination reaction of:



There is a 62% chance that the ozone product in this reaction will be in an excited state.

The *in situ* aircraft measurements of enhanced levels of N_2O associated with thunderstorm lightning reported here verify earlier laboratory experiments and theoretical calculations and give the first direct and unambiguous evidence of the production of a trace gas by atmospheric lightning. Furthermore, recent studies of N_2O reaction kinetics indicate that several reactions leading to the production of N_2O are possible in the energetic electrical environment of the thunderstorm.

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Positive ion composition measurements and acetonitrile in the upper stratosphere

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Although ion chemistry models^{1,2} have predicted proton hydrates (PH) that is ions of the form $H^+(H_2O)_n$, as major ions in the stratosphere, the first *in situ* mass spectrometric measurements^{3–5} revealed another ion family, called non-proton hydrates (NPH). The fractional abundance of these NPH, represented by $H^+X_i(H_2O)_m$, increases from 1 to 90% between 55 and 23 km (refs 1, 5–7). Several proposals^{5,8,9} have been made for the identity of the molecule X, but high resolution spectra¹⁰ and ion abundance measurements^{11,12} suggest that X should be acetonitrile (CH_3CN). This suggestion has been reinforced by laboratory measurements^{13,14} and *in situ* data between 20 and 42 km (refs 6, 7), allowing a determination of the concentration profile of X in this altitude region. Here we report the first positive ion composition data obtained using a balloon-borne instrument between 42 and 46 km altitude. These data extend the density profile of X and give supplementary indications about its identity.

The data were obtained with a mass spectrometer that was flown on 23 September 1982 on a 1,000,000 m³ stratospheric balloon from the CNES launching base of Aire-sur-l'Adour (44° N). Also included in the payload, and mounted above the mass spectrometer, were: a gondola containing photographic equipment for the detection of aerosol layers and an instrument for measuring ozone by means of UV absorption.

The instrument was launched at 12.38 UT and the flight lasted about 7 h. A float altitude of 46 km was reached at 16.00 UT and after sunset the balloon descended slowly to 42 km. The altitude was determined independently from a pressure measurement using a high-precision Baratron gauge and from high resolution radar tracking. The difference between both results was 300 m. Spectra of positive as well as negative ions have been obtained from 46 to 42 km. The negative ion composition data will be reported elsewhere.

The instrument used to obtain the spectra consists of a microprocessor-controlled quadrupole ion mass spectrometer with a mass range of 10–330 AMU and is essentially the same as one that has been described in detail elsewhere^{10,15}. To obtain a greater sensitivity at high altitudes, a larger ion sampling hole (0.4 mm diameter) was used.

Because no major ion groups other than PH and NPH were detected, all positive ion spectra used here were recorded in a moderate resolution mode ($m/\Delta m \approx 17$) that was sufficient to resolve the major ions and their fractional abundance. At some times spectra were recorded in the total ion mode (no d.c. on the quadrupole rods) or with low resolution, which allowed an estimation of the abundance of ions having a mass larger than 256 AMU (ref. 7). To minimize the possible effects of contamination⁷, all useful data were recorded either at float altitude or during the descent part of the flight, with the ion sampling hole pointing downwards.

A typical positive ion spectrum obtained at float altitude is shown in Fig. 1. All major mass peaks below 150 AMU can be

attributed to either PH ions (masses 55, 73 and 91) or NPH clusters (masses 78, 96, ...). Two groups of mass peaks are also present around 278 and 310 AMU. The intensity of these peaks fluctuated with altitude and increased during ascending phases of the balloon altitude oscillations. In spectra recorded during the descent of the balloon, these mass peaks due to contamination were much less pronounced. Laboratory studies in our institute have shown that these ions originated from gases desorbing from the painted surfaces of the photographic payload, heated by solar radiation. These gases appeared to be mainly dibutylphthalate and butylbenzylphthalate vapours; two solvents present in the paint.

As has already been pointed out⁵, the number density of X can be calculated from the observed fractional ion abundances through the continuity equation for NPH:

$$k_1[PH][X] = \alpha[n_-][NPH] \quad (1)$$

where α is the ion-ion recombination coefficient, $[n_-]$ the total negative ion density and k_1 the rate coefficient for the reactions of the type



The total fractional abundances of NPH ions and PH ions, $[NPH]$ and $[PH]$, can be deduced from the ion mass spectra, assuming that ion count rates are reflecting ion abundances. This assumption is believed to be acceptable in view of the moderate resolution used, resulting in low mass discrimination effects.

For k_1 a value of $3 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ was chosen, according to the laboratory measurements of Smith and colleagues¹⁴. The $[n_-]$ was calculated with the parametrization formula of Heaps¹⁶.

For α a parametrization of the form

$$\alpha = 6 \times 10^{-8} \left(\frac{300}{T} \right)^{1/2} + 1.25 \times 10^{-25} [M] \left(\frac{300}{T} \right)^4 \text{ cm}^3 \text{ s}^{-1} \quad (3)$$

was adopted where T is the temperature in Kelvin and $[M]$ the total neutral number density in cm^{-3} . This is a compromise for the different values of α as obtained by recent *in situ* measurements¹⁷, laboratory experiments¹⁸ and theoretical studies¹⁹.

The concentrations of X, thus obtained from spectra similar to Fig. 1 are converted to mixing ratios and represented in Fig. 2 for the different altitudes covered by the present flight.

In the above derivations of $[X]$ using the continuity equation (1), it was assumed that the contaminant gases giving rise to heavy ions (around mass 278 and 310 AMU) do not affect the ambient ion chemistry. To investigate the possible role of contamination in our calculations, we will represent the real number density of X by

$$[X]_R = [X](1 + \epsilon) \quad (4)$$

where $[X]$ is the number density inferred from equation (1). The relative error ϵ will now be calculated for two extreme reaction schemes, which could modify the simple ion chemistry leading to equation (1). In the first one we assumed that the contaminants react with both PH and NPH and give rise to heavy ions CI, which subsequently disappear by recombination. Considering the continuity equation for NPH and CI leads to:

$$\epsilon = k_3[CI](k_2[PH] + k_3[NPH])^{-1} \quad (5)$$

where k_2 and k_3 are common rate constants for the reactions of contaminants with PH and NPH respectively. This implies that the values of $[X]$ would be too low by a factor $(1 + \epsilon)$. The relative error ϵ reduces to zero if the contaminants react with PH only ($k_3 = 0$). When $k_2 \approx k_3$ the error ϵ is estimated to be at maximum 1 in the most contaminated spectra, used for the derivation of $[X]$. This is concluded from estimations of $[CI]/([PH] + [NPH])$ in spectra obtained at very low resolution or in the total ion mode, where few or no errors are induced due to the limited mass range of the instrument. When the

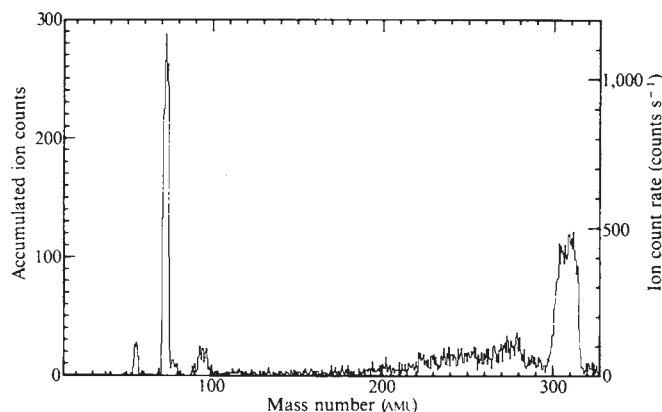


Fig. 1 Typical positive ion spectrum obtained around 45.6 km altitude in the moderate resolution mode ($m/\Delta m \approx 17$). The spectrum has been obtained after 1 scan of 160 s for the mass domain 10–330 AMU. This spectrum has been chosen to show clearly the presence of contaminant ions (mass 278 and 310 AMU). The first part of this spectrum was obtained during a descending phase of the balloon excursion whereas the second half was recorded during ascent.

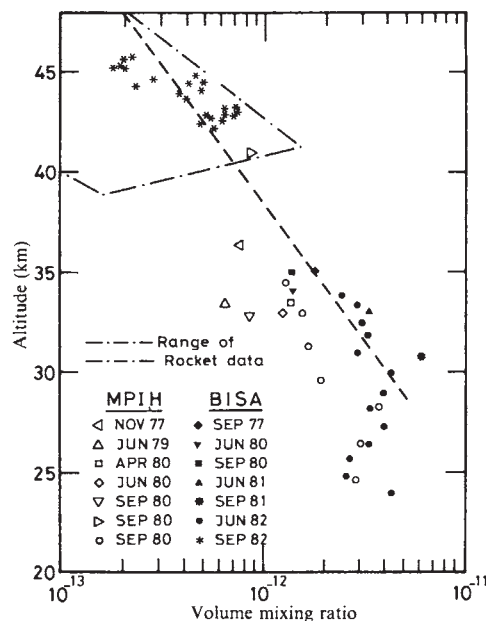


Fig. 2 Volume mixing ratio of X as calculated from ion abundances. Data MPIH were obtained by the group of the Max-Planck-Institut of Heidelberg^{5,6,11}. Data BISA are from Belgian Institute for Space Aeronomy^{4,7,10}. The range of rocket data, obtained by Arnold and colleagues² has been taken from a compilation by Henschen and Arnold⁶. The dotted straight line represents an approximation of the form $f(\text{CH}_3\text{CN}) = 6 \times 10^{-10} \exp(-z/6)$, with z in km.

contaminants are reacting with NPH only ($k_2 = 0$), ϵ can become very large. This would mean that for high values of $[\text{CI}]$ lower values of $[\text{X}]$ should be found. However, spectra obtained during ascending phases of the balloon and showing high $[\text{CI}]$ values seem to indicate the opposite (these spectra were not used for $[\text{X}]$ derivations). This leads us to believe that the first proposed reaction scheme is unlikely.

Another modification of the continuity equation for NPH arises when the contaminant gases react with PH and the resulting CI subsequently react with X to form extra NPH. Continuity considerations now result in:

$$\epsilon = -k_4[\text{CI}](k_1[\text{PH}] + k_4[\text{CI}])^{-1} \quad (6)$$

where k_4 is the reaction rate coefficient of X with CI. Assuming $k_1 \approx k_4$ (which is an extreme case, in view of the large value of k_1) the values of $[\text{X}]$ should be reduced by a factor of 2 in the worst case of contamination for the spectra used, when $[\text{CI}] \approx [\text{PH}]$.

As a general conclusion we feel that it is safe to accept an error of a factor of 2 due to contamination for the values of $[\text{X}]$ as derived in this work. In fact below 44 km where the balloon descended at a rate of 1.3 m s^{-1} , we believe that this error is much smaller, due to the lower outgassing of the optical payload after sunset and due to the induced air flow. This is also confirmed by a total ion mode spectrum, obtained below 44 km during descent, which shows a total abundance of $[\text{CI}] < 10\%$. Furthermore the reasonable agreement of our data points with the rocket data, as observed in Fig. 2, strengthens our faith in their reliability.

Considering the previous remarks and the uncertainty on the quantities α , k_1 and $[n_-]$ used in equation (1), the maximum total error on the data is estimated to be a factor of 3.

When combined with results of previous balloon flights a more complete mixing ratio profile of the molecule X is now obtained. This profile, showing a slow decrease of the mixing ratio of X above 30 km, suggests^{6,7} a source of X below 30 km.

If X were CH_3CN such a source might be surface production by industrial releases or biomass burning (P. Crutzen, personal communication), followed by wash-out, diffusion and photochemical destruction. Recent observations by Becker and Ionescu²⁰ indicate that the concentration of CH_3CN at ground level ranges from 2 to 7 parts per 10^9 by volume, which support the hypothesis of surface emission, followed by strong heterogeneous removal.

For CH_3CN , the emission factors of which are not known yet, the loss processes one expects are reaction with OH and

photodissociation. Since, however, light absorption by CH_3CN and resulting photolysis only start in the far UV (refs 21, 22), the main loss happens through reaction with the hydroxyl radical. Reactions with $\text{O}(^1\text{D})$ and CI may also contribute to the destruction of CH_3CN , although due to the low concentrations of these species and the expected slower reactions this loss term can probably be neglected here. In such a case the steady state continuity equation for CH_3CN can be simplified to

$$\frac{\partial \phi(\text{CH}_3\text{CN})}{\partial z} + k[\text{OH}][\text{M}]f(\text{CH}_3\text{CN}) = 0 \quad (7)$$

with

$$\phi(\text{CH}_3\text{CN}) = -K[\text{M}] \frac{\partial f(\text{CH}_3\text{CN})}{\partial z} \quad (8)$$

where $[\text{OH}]$ is the number density of hydroxyl radicals, k the reaction rate coefficient of CH_3CN with OH, $f(\text{CH}_3\text{CN})$ the mixing ratio and K the eddy diffusion coefficient.

The reaction rate coefficient k , as measured by Harris *et al.*²³, is given by

$$k = 5.86 \times 10^{-13} \exp(-750/T) \text{ cm}^3 \text{ s}^{-1} \quad (9)$$

The total density $[\text{M}]$ can be easily calculated from the ideal gas law and the US Standard Atmosphere. If then we take according to Brasseur *et al.*²⁴

$$K = 1,019 \exp(z/9.43) \text{ cm}^2 \text{ s}^{-1} \quad (10)$$

(z in kilometres), and approximate the mixing ratio $f(\text{CH}_3\text{CN})$ in the altitude region 33–45 km by

$$f = 6 \times 10^{-10} \exp(-z/6) \quad (11)$$

which is the dotted straight line in Fig. 2, the equations (7) and (8) can be solved analytically and the OH mixing ratio can be calculated from the approximated CH_3CN profile.

The result of such a calculation is shown in Fig. 3 for US Standard Atmosphere, autumn temperature conditions. The

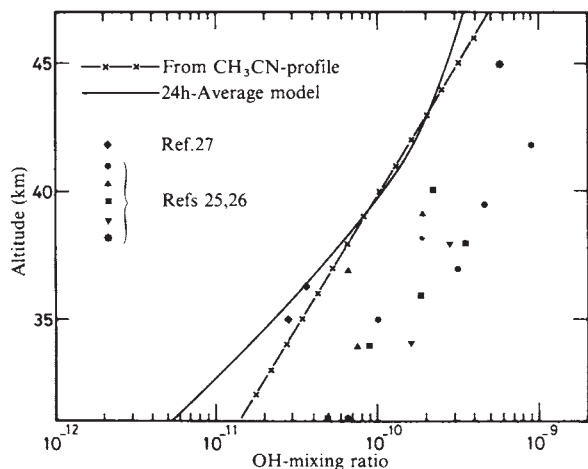


Fig. 3 OH volume mixing ratio profile calculated assuming that X were CH_3CN , and compared with measurements and model calculations. Data for 24-h average model from G. Brasseur (personal communication).

obtained OH profile is compared with recent measurements, performed by different authors²⁵⁻²⁷.

As can be seen the OH mixing ratios of this work are considerably lower than the measurements. Note, however, that the measurements represent instantaneous values of $[\text{OH}]$, whereas the data calculated here are 24 h averages, taking into account the long lifetime of CH_3CN versus the OH reactions (between 400 and 1,000 h for the altitude region under consideration).

In fact, a comparison of the OH profile calculated from the CH_3CN profile with a recent model calculation of the 24-h average of OH according to G. Brasseur (personal communication) turns out to be quite satisfactory, in view of the simple CH_3CN profile assumed (a straight line on semi-log plot). The reasonable agreement of these OH data with previous work can be considered as additional evidence for the identification of X as CH_3CN .

A more complete model of CH_3CN , taking into account possible surface sources, washout in the troposphere, diffusion and photochemical destruction is clearly needed to elucidate this problem. At present, however, the available data seem to indicate that the molecule X is indeed CH_3CN .

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Direct surface imaging in small metal particles

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Atomic-level information about the surfaces of small metal particles has been recorded directly in recent observations with a 600-kV high-resolution electron microscope. Here, we have studied small polycrystalline particles of silver and gold tilted to bring their surfaces parallel to the electron beam. However, unlike previous workers using this normal reflection electron microscopy (REM) configuration, we have used conventional bright field axial imaging thereby considerably facilitating image interpretation. As well as clean, sharp surface images, morphological details of catalytic significance, such as the distribution of surface steps, particle faceting and the nature of surface reconstructions, have been obtained. Moreover, detailed computer simulations confirmed that the electron micrographs can be interpreted in terms of atomic columns and, in particular, established that some micrographs showed, for the first time in a transmission electron microscope (TEM), direct atomic-scale imaging of a reconstructed metal surface.

The first direct atomic imaging in the electron microscope was achieved by Crewe and co-workers using the scanning transmission instrument¹, and several workers^{2,3}, have demonstrated that imaging of atoms is feasible, although less straightforward, in the conventional TEM using tilted-beam dark-field illumination. Good quantitative agreement between experimental bright-field images of isolated tungsten atoms and clusters with calculated contrast levels has also been obtained⁴. Furthermore, it is possible to obtain images of crystals showing surface information on the atomic scale. For example, surface steps in projection have been observed⁵ in MgO and gold⁶ in weak-beam dark-field imaging conditions and also in gold⁷ using forbidden reflections, whilst single atomic steps on the (111) surface of silicon crystals were imaged using a bright field technique⁸. Cowley has used the scanning TEM in a glancing incidence surface imaging mode⁹ to observe unit-cell-high steps on the faces of MgO crystals. This REM technique, which utilizes electrons incident at glancing angles (and thereby produces a severely foreshortened image) has also been used to good effect in TEM studies of surface topography. Direct observations, for example, of superstructures in silicon resulting from surface reconstruction have been made¹⁰. The various configurations and results for surface imaging have been recently reviewed¹¹.

The specimens of silver and gold were prepared by evaporation and epitaxial growth on heated alkali halide substrates, as described elsewhere¹². These were examined at either 500 or 575 kV using the Cambridge University high-resolution electron microscope (HREM)¹³; recent modifications and increased resolution¹⁴ have resulted in almost an order of magnitude improvement in the signal-to-noise ratios for the common lattice spacings (0.235 nm for 111 beams and 0.204 nm for 200) and experimental imaging conditions could be accurately chosen by direct particle observation using an image pick-up

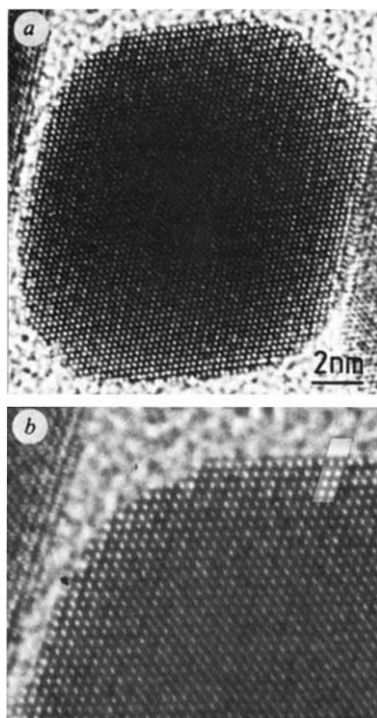


Fig. 1 *a*, High-resolution electron micrograph of a small, slightly-rounded, square-pyramidal particle of gold after tilting from the $\langle 100 \rangle$ epitaxial orientation to the $\langle 110 \rangle$ pole. Visible lattice spacings are 0.235 nm and 0.204 nm. Reverse contrast image—atomic columns appear white. *b*, Enlargement from *a*, with image simulation superimposed. Note visibility of surface steps and faceting.

and viewing system¹⁵. Theoretical calculations, used for confirmation of the image interpretations, were carried out using computer programs based on the multi-slice approach^{16,17}. However, because of the step discontinuity between the finite-thickness crystal and vacuum, as well as periodic continuation effects¹⁸, obtaining a good fit at the edge of the crystal was not simple (L.D.M., in preparation). The standard resolution-limiting factors of energy spread and beam convergence were included by an incoherent image summation in real space rather than the more usual reciprocal space envelope function approach.

In both the experimental and theoretical images we have concentrated, for convenience, on the $\langle 110 \rangle$ pole, although we have some preliminary images which indicate that our results and conclusions are applicable in other projections. Our results are illustrated by Fig. 1, which is an image of a slightly-rounded square pyramid of gold tilted from its original $\langle 100 \rangle$ epitaxial orientation to the $\langle 110 \rangle$ pole. Figure 1*a* shows the entire particle whilst Fig. 1*b* shows an enlargement of its top edge together with the results of a numerical image simulation for a $\langle 111 \rangle$ surface of a crystal of constant thickness. At this particular objective lens defocus (~ 97.5 nm), the white spots in both the calculated and experimental images can be interpreted directly as columns of atoms; surface steps and faceting are clearly visible.

The power of the technique for examining surfaces is well demonstrated by Fig. 2. This image, which was recorded at the optimum defocus (atomic columns black), shows a $\langle 111 \rangle$ surface of a gold particle which has partially reconstructed to a (2×1) surface (that is, a surface with a double periodicity in the $\langle 001 \rangle$ direction). Such reconstructed surfaces are well known from macroscopic surface techniques but have never been imaged directly in previous electron microscopy observations. Although there is some surface roughness, and the reconstruction is irregular, part of the edge of the particle provides unambiguous evidence for the 'missing-row' model for this

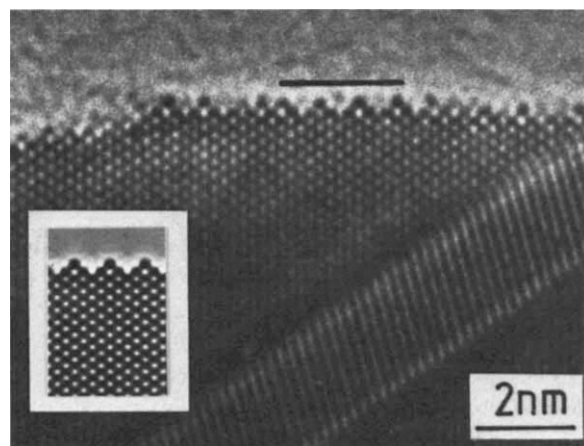


Fig. 2 Small particle of gold showing directly, at the atomic level, a partially-reconstructed (2×1) surface superstructure. Inset shows a calculated image, corresponding to the so-called 'missing-row' model²⁰, which matches well with the experimental image in the region marked.

reconstructed surface¹⁹ (that is, the loss of every second column of atoms on the surface) as shown by comparison with the calculated image inset. Independent confirmatory evidence for this missing-row model has also been recently obtained using scanning tunnelling microscopy²⁰. Further observations and image simulations, which will be described in detail elsewhere (work in preparation), establish that such surface images are indeed sensitive to the chemical nature of the surface; as shown by the inset, for example, we are faithfully imaging the gold surface rather than contamination atoms.

Knowledge of the structure and composition of solid surfaces is essential to a proper understanding of many physical and chemical properties. Moreover, it is the surfaces of small metal particles which are crucial in determining the behaviour of many heterogeneous catalysts. Our results clearly demonstrate that the surface structure of small metal particles can now be directly characterized with atomic resolution in the electron microscope; that is, local structural information is provided which is comparable with the macroscopic results produced by low-energy electron diffraction (LEED) techniques. Admittedly, we have used an idealized model system but other f.c.c. metals are known to have similar particle structure and comparable effects have already been seen in preliminary observations of Pt-impregnated graphite industrial catalysts (E. Noordally and L. A. Freeman, personal communication). Fundamental insights into the structural aspects of heterogeneous catalysis can be expected from a detailed exploitation of this technique.

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Hotspot-spin axis motion or magnetic far-sided effect?

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Palaeomagnetic data from central Pacific sediments and from the Hawaiian–Emperor volcanic chain indicate that, during the Tertiary, either the derived magnetic pole was located alternately on the far and near side of the geographical pole, or that the hotspot coordinate systems (thought to be fixed relative to the Earth's mantle) oscillated relative to the Earth's spin axis.

Our previous determination of the Neogene Pacific plate–spin axis motion¹ used a suite of chronologically overlapping oriented piston cores from the central Pacific basin. About 3,500 discrete determinations of palaeolatitude indicated a fairly constant plate–spin axis motion for the past 15–20 Myr. Comparison of the plate–spin axis motion with the motion of the Pacific plate relative to the hotspot coordinate system suggested that either the hotspots were moving northward relative to the Earth's spin axis, or the palaeomagnetic results were systematically biased with increasing age.

Gordon and Cape² compared Pacific plate–hotspot motion determined from the Hawaiian–Emperor volcanic chain with plate–spin axis motion determined from equatorial sediment facies. They considered a small subset of our earlier data set¹, and attributed the disagreement between that subset of palaeomagnetic data and the hotspot and sediment facies data to the far-sided effect often observed in Tertiary palaeomagnetic data^{3–7}. If all of our previous palaeomagnetic data¹ are considered, however, the disagreement increases with age; and if the disagreement is indeed due to a far-sided effect, then the far-sided effect must increase with age.

If, when averaged over periods of 10,000 yr or more, the Earth's magnetic field is an axially symmetric dipole, then measured palaeomagnetic pole positions (corrected for plate motion) should produce a mean pole at the geographical pole. Wilson and Ade-Hall³ showed that Tertiary palaeomagnetic data generally give a pole position that is on the far side of the geographical pole from the sampling site. Wilson^{4–6} invented a model which explains the far-sided effect by offsetting the dipole to the north of the Earth's equatorial plane along the Earth's rotational axis. This northward displaced dipole makes measured palaeomagnetic inclinations, everywhere on the Earth's surface, more negative than that of a centred dipole; and, therefore, if we assume a centred dipole for pole position or palaeolatitude analysis, calculated virtual geomagnetic poles would be on the far side of the geographical pole from the observer.

Our earlier palaeomagnetic data¹ span the age range 0–22 Myr, although the data are sparse between 15 and 22 Myr. Since the 1979 analysis, additional palaeomagnetic and biostratigraphical data have been obtained from oriented piston cores from the central Pacific basin that now extend the data set back to 49 Myr BP (Table 1). As with our earlier efforts, the post-1979 palaeomagnetic data were obtained using standard procedures⁸. Radiolarians and, in the Oligocene, nanofossils as well, served as stratigraphical guides. The time scale of Ness *et al.*⁹ was used as a chronological reference, in contrast

with our earlier study wherein a previous version⁸ of the palaeomagnetic time scale was used. The palaeolatitudes from the newer cores were derived using Fisherian¹⁰ statistics, whereas the 1979 data were based on inclination alone. Thus, both in terms of time and palaeolatitude analysis, Table 1 contains a heterogeneous data set. For the present discussion, however, this heterogeneity is insignificant since neither the more up-to-date time scale⁹ nor the more rigorous Fisherian approach significantly changes the data in the 0–20 Myr interval. A further consideration is our age resolution, which in the Neogene is now deemed to be of the order of 0.2 Myr, and in the Palaeogene may, at worst, approach 1.0 Myr. A mean dipole palaeolatitude was determined for each core, or core segment, by using palaeolatitudes from between 18 and 165 measured samples (see *N* in Table 1). In two cores, S68-24 and M70-39, Eocene sediments in the bottom portion of the core are separated by a hiatus from the younger material on top; separate dipole palaeolatitudes were calculated for the Neogene and Palaeogene sections of these cores. Our data are not weighted with respect to *N* or net sedimentation rate; thus the number of measurements per time interval may vary between and within cores. The mean age of a core is used rather than the age corresponding to the core's physical (length) mid-point. We judge this biasing to be of negligible effect on the overall trends shown by the data.

The dipole palaeolatitude data (Table 1) provide a measure of the northward motion of the Pacific plate relative to the Earth's spin axis that can be compared with the northward motion of the plate relative to the hotspot coordinate system as determined from volcanic chains on the plate. The palaeolatitude of each core relative to the hotspot coordinate system was determined by backtracking the core to its position at the time the core was deposited (the mean age of each core, or core segment, in this case) using the plate–hotspot poles and rates from work in preparation (D. E. and J. Tuthill). Dipole palaeolatitude minus hotspot palaeolatitude is shown as δ_{lat} in Table 1, and is plotted against time in Fig. 1 where the line is fit by eye. The Neogene data show a progressively increasing difference between dipole and hotspot palaeolatitudes, whereas the data older than 20 Myr indicate that there is a variation in δ_{lat} with time. It is important to recognize that each measured core sample integrates the geomagnetic signal over several thousand years; thus secular variation is averaged out. Note that, in general, the Palaeogene cores are shorter, and thus have fewer measurements than the younger cores. Nonetheless,

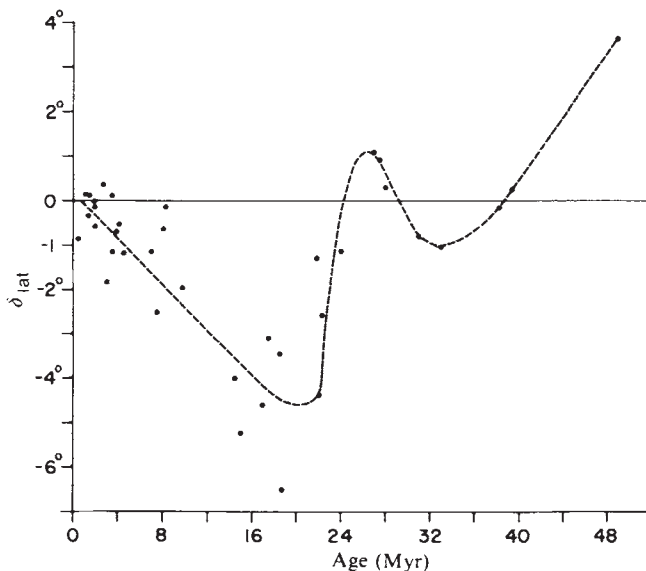


Fig. 1 δ_{lat} equals dipole palaeolatitude minus hotspot palaeolatitude for sediment cores from the central Pacific basin, showing alternating far-sided and near-sided effects and/or hotspot–spin axis motion.

Table 1 Sediment core palaeomagnetic data

Core no.	Lat. (deg)	Long. (deg)	Age range (Myr)	Dipole palaeolat. (deg)	Hotspot palaeolat. (deg)	δ_{lat} (deg)	N	α_{95} *
S68-24	-2.0	-178.8	16.7-27.0	-10.0	-8.7	1.3	120	—
M70-10	1.6	-166.3	16.4-17.5	-8.6	-4.0	-4.6	90	—
M70-14	0.7	-162.5	0.1-0.8	-1.2	-0.3	-0.9	110	—
M70-16	-3.2	-160.3	0.3-3.6	-4.8	-4.2	-0.6	110	—
M70-17	-7.5	161.5	3.7-12.4	-11.1	-10.5	-0.7	112	—
M70-38	-2.3	-171.0	16.4-21	-14.7	-8.2	-6.5	156	—
M70-39	-2.5	-173.3	2.3-4.8	-5.2	-4.1	-1.2	150	—
M70-76	3.0	174.9	12.5-16.4	-6.1	-2.0	-4.1	125	—
K72-36	-2.4	-177.7	16-19	-11.3	-8.2	-3.1	95	—
K72-37	-2.1	-179.0	3.9-11.0	-7.4	-4.9	-2.5	120	—
K72-38	5.4	-164.4	0.0-2.9	-4.4	4.5	0.1	118	—
K72-46	2.3	-164.2	0.6-4.7	1.4	1.0	0.4	120	—
K72-48	4.2	165.8	1.6-5.3	2.8	2.7	0.1	118	—
K76-1-02	-7.6	169.7	0.0-2.7	-8.7	-8.4	-0.3	76	—
K76-1-03	-7.1	171.0	0.4-3.4	8.2	-8.1	-0.2	130	—
K76-1-05	-4.7	173.4	14-16	-15.1	-9.9	5.2	68	—
K76-1-06	-4.4	174.1	3.6-4.6	-6.6	-6.1	-0.5	106	—
K76-1-07	-2.8	177.9	3.0-4.7	-5.1	-4.4	-0.7	101	—
K76-1-08	-2.8	177.9	3.8-4.8	-5.8	-4.6	-1.2	77	—
K76-1-09	-1.1	179.5	1.3-4.8	-4.3	-2.5	-1.8	92	—
K76-1-11	0.9	-178.6	3.0-11.0	-2.9	-1.8	1.2	94	—
K76-1-13	2.7	-178.7	0.2-2.1	2.1	1.9	0.2	105	—
K76-1-14	2.9	-178.5	5.3-11.2	-0.3	-0.2	-0.1	83	—
K76-1-15	2.9	-178.5	0.4-3.3	1.9	1.9	0.0	157	—
K76-1-16	4.6	-176.5	13-24	-4.8	-1.3	-3.5	140	—
K76-1-17	4.6	-178.6	8.0-11.5	-0.9	1.1	-2.0	165	—
K72-39	8.5	-116.9	29-34	-1.7	-0.9	-0.8	62	2.96
K78-0-20	9.2	-170.8	25.0-29.2	2.1	1.0	1.1	48	5.07
K78-5-23	9.2	-170.7	29.5-34.5	2.0	-0.9	-1.1	74	2.58
K78-0-23	9.1	-170.7	25-29	1.7	0.8	0.9	84	4.85
K78-0-12	11.4	-168.2	48-50	-2.1	-5.7	3.6	59	5.12
K72-42	7.2	-166.2	22-27	-1.1	0.0	-1.1	90	3.62
K78-0-15	9.8	-169.1	24-32.3	1.6	1.3	0.3	39	4.43
K78-0-16	9.8	-169.1	20.5-24	0.5	3.1	-2.6	57	3.20
K78-0-17	9.8	-169.1	20-24	-1.2	3.2	-4.4	72	7.34
S68-24	-2.0	-178.8	38-39	-13.9	-13.7	-0.2	18	—
M70-39	-2.5	-173.3	38.5-40.5	-14.4	-14.5	0.1	56	—

* See text.

even though some of the older data, especially the two Upper Eocene cores, may be less reliable than the Neogene data, the variation in δ_{lat} shown in Fig. 1 is considered to be real, particularly in view of the agreement from one core to the next.

Sager¹¹ has determined a dipole palaeolatitude for Abbott Seamount in the Hawaiian-Emperor chain by analysis of a marine magnetics survey of the volcano, and has compiled the palaeomagnetic data from other volcanoes in the chain. The Hawaiian-Emperor volcanoes were formed by a hotspot that is presently located approximately under the island of Hawaii; thus all of the Hawaiian-Emperor volcanoes should have been formed at the same hotspot palaeolatitude. For a consistent comparison with the palaeomagnetic data set, however, the hotspot palaeolatitude for these volcanoes should be determined by rotating the volcanoes back in time using the same plate-hotspot poles and rates. The hotspot palaeolatitudes determined by these rotations obviously depend on the ages assumed for the volcanoes. If we use the ages given by Dalrymple *et al.*¹², and rotate the volcanoes back to where they would have been at that time, we find that some hotspot palaeolatitudes are considerably different from the present latitude of the Hawaiian hotspot near 19.4° N. This is particularly apparent in the case of Ojin and Suiko which yield hotspot palaeolatitudes of 15.93° N and 16.55° N, respectively. These discrepancies could be due to motion of the Hawaiian hotspot not represented by the plate-hotspot poles and rates, or to age dates that are not representative of the true age of the volcano. To avoid these difficulties, we assume that the rate of propaga-

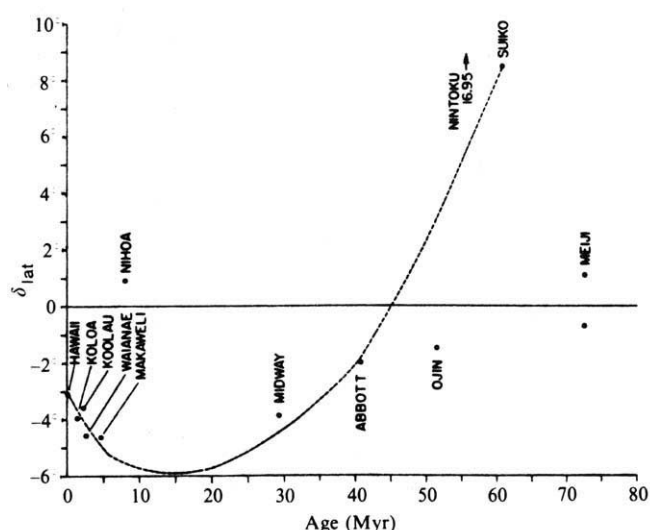


Fig. 2 δ_{lat} equals dipole palaeolatitude minus hotspot palaeolatitude for the Hawaiian-Emperor volcanic chain.

tion of volcanism along the Hawaiian-Emperor chain has been constant with time. A straight line was fit to the age versus distance data using the age data from Dalrymple *et al.*¹² and M. Garcia (personal communication), and the distance from each edifice to the hotspot (Loihi) measured along small circles

Table 2 Hawaiian-Emperor palaeomagnetic data

Volcano	Lat. (deg)	Long. (deg)	Age (Myr)	Dipole palaeolat. (deg)	α_{95}	N	Hotspot palaeolat. (deg)	δ_{lat} (deg)	Ref.
Hawaii	19.50	-155.32	0.35	16.3	1.9	129	19.4	-3.1	17
Koolau	21.41	-157.76	2.6	16.8	3.6	33	19.4	-2.6	18
Waianae	21.51	-158.14	3.7	15.7	3.3	55	19.4	-3.7	18
Koloa (Kauai)	22.10	-159.54	1.4	17.3	3.0	19	19.4	-2.1	19
Makawela (Kauai)	22.10	-159.54	3.75	15.6	3.1	24	19.4	-3.8	19
Nihoa	23.13	-161.79	7.2	21.0	6.6	14	19.4	1.6	20
Midway	28.23	-177.37	27.7	15.4	5.4	13	19.4	-4.2	21
Abbott	31.76	174.28	42.5	17.5	4.2	—	19.4	-1.9	11
Ojin	37.77	170.38	55.2	17.6	13.2	6	19.4	-1.8	22
Nintoku	41.00	170.58	56.2	36.0	24.6	4	19.4	16.6	22
Suiko	44.57	170.32	64.7	26.9	3.5	65	19.4	7.5	22
Meiji	52.97	164.55	>70	19.2	4.1	6	19.4	-0.2	23

about the poles. The correlation coefficient for one line fit to the age versus distance data was larger than that for lines fit to various subsets of the data; thus, within the limits of the data, the rate of propagation of volcanism has been constant with time. The 'adjusted age' that each volcano would have if its age was on the least-square line fit to the whole data set was then used to backtrack the volcano to determine the hotspot palaeolatitude. The variations in hotspot palaeolatitudes calculated in this way result from the size of the hotspot and the uncertainty in the present location of the hotspot. Uncertainties in determining plate-hotspot pole positions and rates have the same effect on both of the data sets discussed here. Use of different poles and rates changes the values of δ_{lat} calculated but not the pattern of variations of δ_{lat} . For example, using the plate-hotspot pole of Turner *et al.*¹³ rotates the curve, but does not change the shape of the curve.

Figure 2 is a plot of δ_{lat} against 'adjusted age' for the Hawaiian-Emperor data set. While the Hawaiian-Emperor data do not show the oscillation between 22 and 36 Myr BP that occurs in the central Pacific basin data set (Fig. 1), a long-period change in δ_{lat} occurs in both.

The central Pacific basin palaeomagnetic data now show that δ_{lat} has undergone an aperiodic variation in the past 49 Myr. The Hawaiian-Emperor data set, which consists of 368 measurements on lava flow samples and one inclination measurement from a magnetics survey, also appears to support a long term change in δ_{lat} . The Hawaiian-Emperor data fail, however, to corroborate a high-frequency oscillation in δ_{lat} between 22 and 36 Myr BP, although Midway has an age in this range. The dipole palaeolatitude for Midway was determined from only 13 measurements and secular variation may not have been averaged out. The other Hawaiian-Emperor δ_{lat} calculated from <15 measurements (N in Table 2) disagree with the trends shown by the more reliable data. For example, the δ_{lat} calculated for Nihoa is significantly different from the pattern established by the younger values. For the same reason, we also consider the δ_{lat} for Ojin, Nintoku, and Meiji to be dubious.

Morgan¹⁴ compared the past 200 Myr of motion of the continents relative to the hotspot coordinate system with the motion of the continents relative to the spin axis as determined from palaeomagnetic data. He suggested that the mantle (and hotspots) have made large rapid shifts relative to the spin axis followed by periods of little or no motion. He found that during the period 50–5 Myr BP little motion occurred. Harrison and Lindh¹⁵ found approximately 20 degrees of motion between the hotspot and geomagnetic coordinate systems during the past 200 Myr, with a relatively small amount of that motion occurring between 70 and 20 Myr BP. From these studies it appears that the time spanned by the central Pacific basin palaeomagnetic data presented here was a period of little or no hotspot-spin axis motion.

The short-term variations in δ_{lat} could be superimposed on long-term hotspot-spin axis motion, and the variations may not be apparent in the other palaeomagnetic data sets due to the sparsity of measurements in these data sets. The data presented here contain >3,600 measurements spanning the past 49 Myr. The only equivalent data set in the Pacific, in terms of data homogeneity and density, is that obtained from an analysis of the skewness of marine magnetic anomalies by Jarrard and Cande¹⁶. Their data set contains >1,200 points, and shows variations in general agreement with those observed in δ_{lat} .

The changes in δ_{lat} shown by the central Pacific basin and Hawaiian-Emperor data (and by the skewness data¹⁶) could be caused by either: (1) the hotspots moving in some oscillatory manner about the Earth's spin axis; (2) non-dipole components in the Earth's magnetic field varying with time and producing what appear to be alternating far-sided and near-sided effects, or (3) both. The data are insufficient to decide among these possibilities; however, some form of oscillation is characteristic of secular variation, dipole wobble, and field reversals⁵, so variations in non-dipole components are not unexpected. Also, the changes in δ_{lat} observed in the central Pacific basin data would require very rapid accelerations of the core relative to the mantle if the hotspots were to move relative to the spin axis. Thus we think that the observed changes in δ_{lat} are most likely to be caused by changes in the Earth's magnetic field.

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Seismicity of Yemen

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The Arabian Peninsula often appears totally aseismic on maps of world seismicity, reflecting the apparent absence of earthquakes during the twentieth century and particularly since the improvement of the worldwide seismological network after the 1960s. Events in south-west Arabia have seldom attracted attention outside the area and this has hindered reporting of earthquakes in regional catalogues. We show here that by contrast, historical sources of local information describe a fairly continuous occurrence of medium magnitude, damaging shocks over the past 12 centuries, including a destructive event in 1941 that in many respects resembles the recent earthquake of December 1982. The common presumption that the Arabian Peninsula has a very low seismicity is incorrect.

The earthquake of 13 December 1982 in north Yemen resurrected the question of how little is known of seismic hazards in certain parts of the world. The main shock, at 1309 (GMT), of magnitude $M_s = 5.7$, occurred near 14.65°N – 44.05°E , affecting the rather densely populated Dhamar district of the Yemen, in which 280 settlements were ruined. About 1,900 people were killed, mostly in rubble masonry two-storey houses, and about 20% of the district's population became homeless. Material losses amounted to £135 million. Much of the destruction was due to the high vulnerability of the local types of dwellings; the very few better-built houses in the area suffered little or no damage. The shock was followed by numerous and widely scattered aftershocks which added to the damage. Earthquake-related extensional ground cracks were found in the epicentral region running for a few hundred metres to more than 10 km in length, trending north-northwest.

The few previous earthquakes instrumentally located along the coastal plains and western margin of the Arabian plate inland of the escarpment, as compared with the much larger number of events originating offshore in the Red Sea Axial and Main troughs, give a misleading impression of low seismicity in the land areas. In contrast, the long-term documentary record corrects this bias in that it reveals evidence of significant seismicity in the coastal and inland regions of south-west Arabia.

Figure 1 shows the very approximate epicentral locations of the most important historical earthquakes in our files. These go back to the eighth century AD and include the little-known destructive earthquake of 1941, an event in many respects similar to the 1982 earthquake. In the absence of satisfactory regional catalogues, data have been retrieved from primary sources including Arabic histories and local chronicles, narratives of passing travellers and, more recently, British official archive material. One of the main problems encountered in analysing accounts of earthquakes in the region is the identification of many of the places mentioned as affected; this is partly because of uncertain orthography, partly because some places may no longer exist or do so under a different name, and partly because of the unavailability of adequate maps. Locations of historical events on Fig. 1 therefore depend closely on the positions of the ancient centres of population. We have omitted earthquakes felt in the Yemen which most probably originated offshore in the Red Sea or in the Afar, as well as volcanic eruptions, meteoritic impacts and landslides. Some of the latter, as in 857, 1255, 1400 and 1583 are reported as earthquakes by contemporary authors. The only shock in our area of interest that is mentioned by Sieberg¹, on 22 February 1400, was caused by meteorite impact².

The earliest known earthquake occurred in 742 in the 'desert of Saba'—probably somewhere round the edges of the desert

between Shabwah (in the Hadramawt) and Marib. Many villages were overwhelmed by collapsing mountains^{3–5}. This should have been a relatively large event as its occurrence is noted by near contemporary chroniclers writing in Constantinople and Upper Mesopotamia.

The first adequately recorded event occurred in 827, when the districts of both Aden and San'a were affected; the shock destroyed houses and villages and caused many deaths⁶.

In 1072 a great earthquake in San'a, Zabid and Mukha destroyed houses, killing about 50 people⁷. This was followed by another earthquake on 11 September 1154 which occurred between San'a and Aden, destroying many villages, castles and dwellings. In San'a alone, 300 people were killed and more in the villages outside^{6,8}. A century later, on 22 November 1259, a slight shock was felt at San'a causing no damage; this was followed on 10 December by another earthquake which caused damage to many places in the mountains west of the town². More shocks were felt in San'a in 1265⁸.

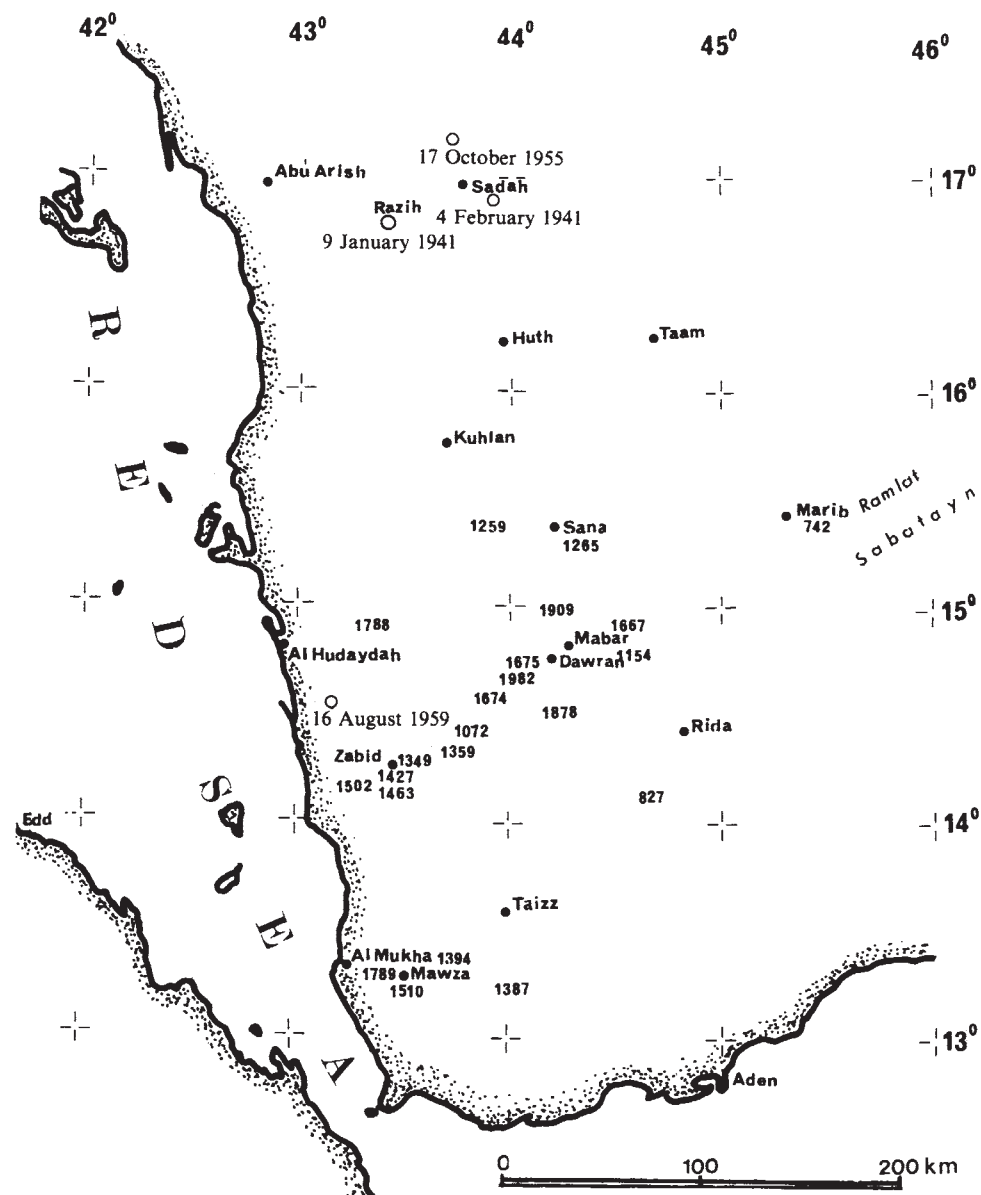
In November 1349 an earthquake in Zabid caused panic but apparently no damage⁷. Ten years later, in 1359, a great earthquake affected Zabid, San'a and Aden. Shocks continued intermittently from midday to early evening and many houses were destroyed. This event affected a large area: 51 people were killed in Zabid and a few in San'a and Aden⁷. In September 1387 an earthquake destroyed many houses in Aden, without loss of life. This shock, which was followed by many others that lasted several days, caused great damage in Hajar and in other places not at present identified^{2,7}. During the period March–April 1394 there was a sequence of about 40 shocks that were felt in the region of the town of Mawza^{2,7}.

Zabid was again shaken in 1427. The main shock and numerous aftershocks also affected other places and many houses were destroyed. About 60 people were killed⁷. Again in 1463 a series of earthquakes occurring over a period of three days destroyed 50 houses in Zabid, killing 10 people⁷. A further sequence of shocks in 1502–3 caused panic in Zabid but no damage. Two years later an earthquake near Zayla' in Somalia was also strongly felt in Zabid, and further shocks were reported there in 1509 and 1510. The most serious of these probably originated in the region of Mawza', where a series of large and small shocks were reported. These caused great losses to the people, as no house escaped damage and the poorly constructed ones suffered extensive fissuring. Cracks opened up in the cultivated fields and all the wells became muddied⁹.

A great earthquake is reported in Aden in 1613 that made the ground swell in waves—probably from an offshore epicentre⁷. Later on 22 September 1644, a strong tremor is reported to have caused rockfalls at a place called al-Ashshah¹⁰. The region affected is east of Sa'dah, although there was also a village of the same name in the Dhamar district. This event may have been a landslide rather than a genuine earthquake. Further shocks were felt at San'a and elsewhere in 1647 and again in March 1667. The latter earthquake damaged houses and was felt throughout most of the Yemen. Of particular interest are the shocks of August 1674, about 30 in all, affecting Dawran and culminating in a major earthquake in 1675 that split many houses, including Dar al-Hasin and caused rockfalls from Jabal Dawran¹¹. This earthquake, which was felt in San'a, happened in the epicentral region of the recent earthquake of 1982. Details are said to be found in the *Bahjat al-zaman*, a continuation of the *Inba al-zaman* (see ref. 8) by Yahya b. al-Husayn (d. 1689). No copies of this work exist outside Yemen and we have yet to verify this information.

Another large earthquake occurred in 1788 in the region of Hudaydah (Hodeida) affecting several places, such as Bayt Qasr and Bayt Hindi, where houses and buildings were destroyed. At one place the ground was fissured and fire caused further damage. The shock, which happened at the end of November and was followed by other shocks to the end of July 1789, was strongly felt from Mukha in the south to Abu 'Arish in the north, reported from many coastal towns whose identification is uncertain¹². The shocks of 1789 were strongly felt at Mukha

Fig. 1 Historical seismicity of the Yemen. Years indicate approximate location of epicentral regions affected by damaging or destructive earthquakes. Open circles show teleseismic location of twentieth century events.



where they completely destroyed a small town in the mountains, 30 km inland¹³.

Early in January 1859 Aden and Mukha and the intervening areas were shaken by a series of shocks, probably from an offshore earthquake¹⁴. Another earthquake is reported in 1873 in the region of al-Haymah, west of San'a, which may not be a genuine seismic event; a large landslide from a mountain near Bayt al-Nash diverted the course of the river and did much damage¹⁰. Five years later, during the summer of 1878, there was a long sequence of earthquakes and tremors in the towns of Dhamar and Yarim and in their respective provinces. Shocks lasted for three months up till August and destroyed many houses¹⁰. These events occurred in much the same region as was affected by the 1982 earthquake. On 2 August 1895 a series of three shocks occurred at Mukha and Ta'izz; it is not known whether any damage was caused^{10,15}.

In 1909, a destructive earthquake is reported in Bilad al-Waynan (Wa'lan?). There are said to have been 300 people killed and 400 houses were destroyed¹⁰. The date given is April-May, but there may be some connection with a shock reported in Djibouti on 19 February 1909¹⁶.

The International Seismological Summary gives a rough teleseismic location of an earthquake on 4 October 1924 at 15°N–44°E, in the Dawran Anis area. We have not so far found any macroseismic information for this event, which has most probably been mislocated.

Preceded by a foreshock on 9 January 1941, an earthquake on 11 January 1941 caused widespread damage in north Yemen. Using 84 and 74 stations respectively ISC and Ambraseys have relocated the earthquake at 16.64°(±0.1)N–43.31°(±0.05)E. The origin time is 08 h 31 min 59 s (±0.7) and the surface wave magnitude $M_s = 5.8$ (±0.3) from eight stations. The relocated epicentre of the main aftershock on 4 February at 09 h 17 min 50 s (±5.5) was found at 16.90°(±0.65)N–43.90°(±0.37)E from 15 stations, with a magnitude $M_s = 5.2$. For both solutions there are no observations from stations between azimuth 90°–270° which has probably had the effect of dragging the hypocentre northwards by possibly as much as half a degree or more.

The 1941 earthquakes affected a large area, the main shock being felt from Jizan to as far as al-Mukalla (in south Yemen). At Razih several settlements were destroyed with small loss of life and landslides blocked the road at the head of the Razih valley. Damage extended as far south as Kuhlan and Hajjah. Two strong aftershocks occurred at 0918 on 4 February and at 1903 GMT on 23 February, of which the former had a magnitude $M_s = 5.2$. These were reported from Hudaydah, Bayt al-Faqih, al-Sa'id and Bura' and caused additional damage. Aftershocks continued up to the second week of March. It is reported that altogether 1,200 people were killed and 200 wounded in these earthquakes, which totally destroyed many houses and damaged about 1,400¹⁷.

On 17 October 1955 at 2008 GMT another earthquake of magnitude $M_s = 5.0$ occurred in north Yemen; this was widely felt but macroseismic information is not sufficient to locate the event. Its teleseismic location is rather poor, placing it north of Sa'dah¹⁸. Due to a misprint in (ref. 19), this event has been duplicated for the same date in 1955 and 1965 in all regional data banks.

There is no macroseismic data for the relatively small earthquake of 16 August 1959 at 1331 GMT, which is located teleseismically near Bayt al-Faqih¹⁸.

The data currently at our disposal therefore indicate that destructive shocks have occurred in the Yemen with some regularity over the past 1,200 years. All these events have been of medium magnitude, most of them followed by long after-shock sequences, a common characteristic of earthquakes associated with volcanism. The location and size of many of these shocks are at present uncertain, but it is possibly of significance that Fig. 1 hints at a north-east-south-west trend in the distribution of shocks through the Dhamar region and that the recent earthquake fills a gap in the historical pattern. Further research on local sources of information and field studies of Quaternary geology of the Yemen should throw light on these issues. More work on the neotectonics of south-west Arabia will be needed before we can arrive at a more accurate assessment of the regional seismicity of the area.

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Evidence for dyke intrusion earthquake mechanisms near Long Valley caldera, California

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A re-analysis of the magnitude 6 earthquakes that occurred near Long Valley caldera in eastern California on 25 and 27 May 1980, suggests that at least two of them, including the largest, were probably caused by fluid injection along nearly vertical surfaces and not by slip on faults. Several investigators^{1,2} have reported difficulty in explaining both the long-period surface-wave amplitudes and phases and the locally recorded short-period body-wave first motions from these events, using conventional double-couple (shear fault) source models. They attributed this difficulty to: (1) complex sources, not represent-

able by single-fault models; (2) artefacts of the analysis methods used; or (3) effects of wave propagation through hypothetical structures beneath the caldera. We show here that the data agree well with the predictions for a compensated linear-vector dipole (CLVD) equivalent-force system³ with its principal extensional axis horizontal and trending N 55-65° E. Such a mechanism is what would be expected for fluid injection into dykes striking N 25-35° W, which is the approximate strike of numerous normal faults in the area.

Given *et al.*² studied the mechanisms of three of the four largest earthquakes of 25 and 27 May 1980 (Fig. 1, events 1, 3 and 4). They inverted the amplitudes and phases of long-period (150 s and 197 s) surface waves recorded by the Global Digital Seismograph Network to obtain a moment-tensor representation of the largest event (1633:44 UTC 25 May 1980), imposing the constraint that there be no net volume change: $M_{xx} + M_{yy} + M_{zz} = 0$. Because the earthquake was near the free surface, the moment-tensor components M_{xz} and M_{yz} (the z axis is taken to be vertical) could not be resolved and were also constrained to be zero. The result was a non-double-couple mechanism whose principal-axis representation is a combination of a vertical compressional force-dipole (moment $M = 1.33 \times 10^{25}$ dyn-cm), a horizontal compressional dipole oriented N 22° W ($M = 1.35 \times 10^{25}$ dyn-cm), and a horizontal extensional dipole oriented N 68° E ($M = 2.68 \times 10^{25}$ dyn-cm). Within the measurement error, this mechanism is a CLVD. Given *et al.* sought to explain this result by adding M_{xz} and M_{yz} components to the moment tensor to obtain a conventional double-couple force system, which they interpreted as left-lateral oblique normal slip on a fault plane striking N 12° E and dipping 50° E. The two smaller events analysed (1955:51 UTC 25 May and 1450:57 UTC 27 May), did not generate usable surface waves at periods suitable for accurate application of the same inversion scheme, and so, instead, Given *et al.* used a relative inversion technique based on shorter period (80 s) waves to compare these two smaller events with the first event. The resulting mechanisms were similar but involved smaller components of normal faulting.

The double-couple mechanisms proposed by Given *et al.* are consistent with the sparse long-period teleseismic P-wave first motions available as well as with the observed surface waves. However, as Cockerham *et al.*⁴ and Given *et al.*² noted, they are inconsistent with the numerous short-period first motions observed in California and Nevada (Fig. 2). CLVD mechanisms like the one derived from surface waves, however, are consistent

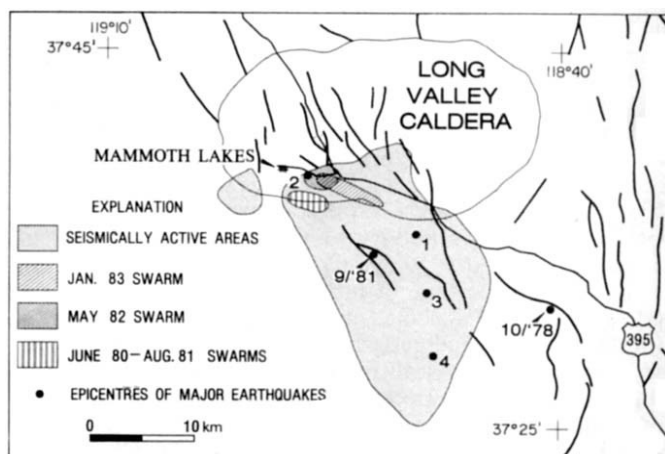


Fig. 1 Long Valley caldera and vicinity, showing area of seismic activity and locations of earthquake swarms. Heavy lines: normal faults. Epicentres of magnitude 6 earthquakes of 25 and 27 May 1980: 1, 1633:44 UTC 25 May; 2, 1649:27 UTC 25 May; 3, 1944:51 UTC 25 May; 4, 1450:57 UTC 27 May. Also shown are large earthquakes of 1642:48 UTC 4 October 1978 and 1153:26 UTC 30 September 1981, US highway 395, California highway 203 and town of Mammoth Lakes.

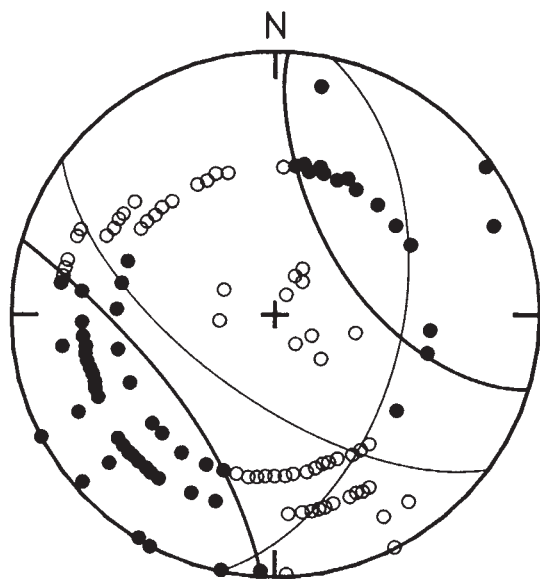


Fig. 2 Short-period P-wave first motions (from Fig. 5b of ref. 2) for earthquake of 1633:44 UTC 25 May 1980. Lower focal hemisphere is shown in equal-area projection. Solid circles, compressions; open circles, dilatations. Light curves, nodal planes for shear fault with strike 12°, dip 50°, and rake -35°, as proposed by Given *et al.*²; heavy curves, nodal lines for moment tensor fitted to these data, which is close to a pure CLVD.

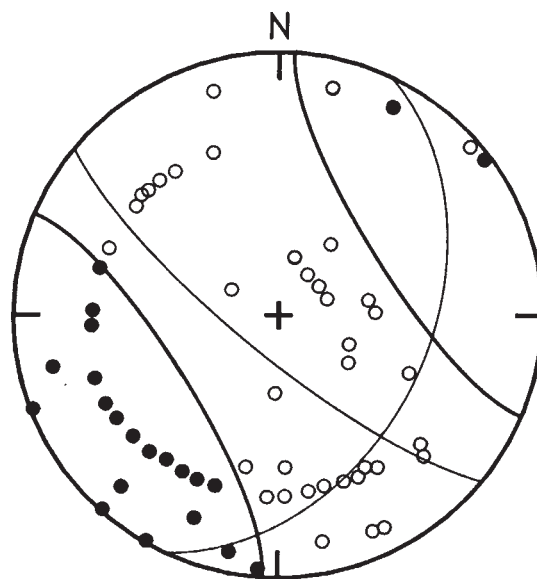


Fig. 3 Same as Fig. 2 for earthquake of 1450:57 UTC 27 May 1980. Data from Fig. 10 of ref. 2. Shear fault has strike 25°, dip 42°, and rake -19°. Heavy curve is for hand-fitted CLVD with horizontal primary axis trending N 58° W.

with the first-motion data. Figure 2 (from Fig. 5b of ref. 2) shows the short-period P-wave first motions for the first and largest earthquake (1633:44 UTC 25 May), and the nodal planes for their mechanism. Also shown are the nodal surfaces (no longer planes) for a general dilatation-free moment-tensor source fitted to these data, using the technique described by Julian⁵. This mechanism (principal moments in the ratios 2:-1.2:-0.8, with the primary axis extensional and trending N 55° E and plunging 7.5°) is an almost pure CLVD, and is similar to the mechanism obtained from surface-wave inversion before the elements M_{xx} and M_{yy} were added to it. Figure 3 (from Fig. 10 of ref. 2) shows similar data for the event at 1450:57 UTC 27 May. The mechanism shown was fitted by hand and is a pure CLVD with its primary axis horizontal and trending N 58° E. As can be seen, the CLVD mechanisms fit the short-period first motions much better than do the double couples: they have only 4 and 2 inconsistent data for the two events, whereas the double couples have 32 and 16.

The CLVD may be thought of as a point-force-system without net force or torque that has no explosive, implosive, or double-couple component. In the principal-axis coordinate system, it consists of three orthogonal force-dipoles with moments in the ratio 2:-1:-1. By way of contrast, a double couple has principal moments in the ratio 1:0:-1. CLVD earthquake mechanisms were proposed 50 yr ago by Ishimoto⁶, who called them 'conical sources' because of the configuration of the P-wave nodal surfaces, and attributed all earthquakes to sudden magma intrusion. (Aki⁷ has discussed the subsequent controversy in the Japanese seismological community.) Knopoff and Randall³ proposed CLVD as a mechanism of deep-focus earthquakes, but a study of P-wave amplitudes from five such earthquakes⁸ failed to prove the hypothesis. Until now, the CLVD had remained a curiosity, not forbidden by Newton's laws but never definitely observed.

Injection of a fluid into a crack would be expected to generate earthquakes with CLVD mechanisms. The point-force system equivalent to a tensile crack in an isotropic medium consists of three mutually orthogonal dipoles, with moments in the ratio $(\lambda + 2\mu) : \lambda : \lambda$, where λ and μ are the Lamé elastic moduli, and with the strongest (first) dipole oriented in a direction normal

to the crack⁹. At depths of more than a few kilometres, rocks cannot sustain the stress differences that would accompany the creation of a void, and a tensile crack can form only if a fluid under high pressure flows into it. Volume is conserved in such a process (at least approximately, neglecting such phenomena as thermal expansion, melting and freezing). Thus, in the Earth, the triple of dipoles must be accompanied by another force system with an implosive component that just cancels out its explosive component. If this second mechanism is symmetrical about the vector direction normal to the crack, the resulting force system is a CLVD.

We can estimate the volume of fluid injected from the seismic moment of the associated earthquakes if we make an assumption about the equivalent-force system representing flow into the dyke. If a pair of equal dipoles in the plane of the crack is appropriate, we obtain

$$V = \frac{1}{\lambda + 2\mu} M$$

where M is the moment of the extensional dipole. Alternatively, the assumption that the fluid flow is equivalent to an isotropic implosion gives

$$V = \frac{3}{4\mu} M$$

Taking $\lambda = \mu = 3 \times 10^{11}$ dyn cm⁻² and using the first assumption, the surface-wave moment of the largest event gives $V = 0.03$ km³. This value is a lower bound; the second assumption gives a value 2.25 times as large. These values are an appreciable fraction of the volume (0.15 km³) deduced from geodetic-uplift data by Savage and Clark¹⁰ for resurgence of magma into a chamber in the caldera some time between 1975 and October 1980. The inferred position of this chamber is about 6 km north of the north end of the seismically active area and 11 km beneath the surface. Clearly, the dyke injection and the caldera filling are related to each other, although the nature of this relation—whether the dykes fed the magma chamber or radiated from it—is unclear.

The dyke-injection model may also agree better with the horizontal deformations reported by Savage and Clark¹⁰ than do faulting models. Points east of the caldera and the seismic zone moved eastward between July 1979 and October 1980, though not precisely as expected for expansion of a buried magma chamber—the observed displacements were slightly too large and had an additional northward component. Dyke injection accompanying the earthquakes south of the caldera would cause displacements in the correct directions to account for the discrepancies. The one trilateration station south of the caldera and west of the earthquakes moved slightly to the north during the same period, whereas magma-chamber expansion would lead one to expect a large southward displacement. The assumption that the earthquakes involved left-lateral shear on north-south-trending faults makes the discrepancy even worse, unless the fault dips shallowly and surfaces west of the trilateration station. Dyke injection, on the other hand, does not have this difficulty and may be able to generate the needed northward motion. J. C. Savage (personal communication) is undertaking a quantitative re-evaluation of the geodetic data in the light of the dyke-injection hypothesis.

The evidence presented here for fluid injection in and near Long Valley caldera reinforces several other lines of evidence that recent earthquakes there are related to igneous processes: The duration of the seismic activity (1978 to the present), the migration of earthquakes over time, the swarmlike behaviour of the seismicity, and the geodetically observed uplift¹⁰. Such an interpretation must now be considered highly probable. Moreover, it may be that similar earthquake mechanisms have escaped notice in other volcanic areas.

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Measurements of iron(III)-rich fayalites

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Recent measurements are reported here of the Mössbauer spectra of selected fayalites (members of the olivine group) from various localities, which indicate large amounts of iron(III) in several of the samples. These features in the spectra are not attributable to magnetite or haematite contamination, but are due to Fe³⁺ ions contained in the fayalite crystal structure. Such data are contrary to the generally held belief that fayalite is incapable of accommodating much iron(III) in its crystal structure. This discovery may have severe implications for the thermodynamics of the FeO–SiO₂ system, as well as for the thermal conductivity of olivine.

The possibility of the existence of iron(III) in fayalite was suggested by the recent discovery in the People's Republic of China of an iron silicate mineral called laihunite^{1–3} or ferrifayalite⁴, combined with our observation of an anomalous four peak Mössbauer spectrum of fayalite from the Mourne Moun-

tains in Ireland. Laihunite(Fe_{1.00}³⁺Fe_{0.58}²⁺Mg_{0.03}Si_{0.96}O₄) is a black mineral closely related to fayalite in structure and composition, but containing both Fe³⁺ and Fe²⁺ ions, with actually more of the former than the latter. The crystal structure of laihunite is similar to that of normal fayalite, but ordered cation vacancies lower the space group from Pbnm in fayalite to P2₁/c in laihunite. Cation vacancies in laihunite are necessary to balance the charge of the crystal. Laihunite also has a distinctive IR spectrum.

Previous discoveries of iron(III)-bearing fayalites have been made in the Soviet Union^{5,6}. These minerals, talasskite and ferrifayalite (apparently not the same as the Chinese ferrifayalite), are unconfirmed to be true minerals. Talasskite was discovered in 1934, and to my knowledge has not been studied since, although its analysis has been listed⁷. Ferrifayalite was believed even by its discoverers to be not a single mineral, but a metastable 'mineral' consisting of three intimately intermixed phases: fayalite, and thinly dispersed forms of haematite and silica.

Samples of dark coloured to black fayalites were obtained for study from several museum collections. These samples included fayalites from Massachusetts, Ireland, Italy, Colorado, Sweden, Antarctica, and an artificial fayalite from slag. In addition, a sample of ferrifayalite (lahunite) from China was examined. Of these samples, four (including ferrifayalite), were found to contain significant amounts of iron(III), as determined by Mössbauer spectroscopy. See Table 1 for the geological setting of these minerals. The iron(III)/(II) ratios of these samples range from 0.19 to 1.38 (for ferrifayalite itself).

Mössbauer spectra were taken on an Austin Science Associates spectrometer. The γ -ray source consisted of ⁵⁷Co diffused into either Pd or Rh foils having activities of 50–100 mCi. In general, baseline counts exceeded 1 million. Spectra were calibrated against metallic iron foil standards. Iron in the samples was constrained to be between 5 and 10 mg cm⁻². Fitted spectra of four iron(III)-rich fayalites and one 'normal' fayalite (Rockport, Massachusetts) are shown in Fig. 1. Mössbauer parameters of normal and iron(III)-rich fayalites are summarized in Table 2.

The spectra of the iron(III)-rich fayalites each show two doublets assigned to octahedral Fe²⁺ and Fe³⁺ ions. Expanded velocity spectra of all fayalites were also taken to eliminate the possibility of magnetite or haematite contamination. The centre shift and quadrupole splitting of the iron(III) doublet are consistent with the idea of iron(III) in the fayalite matrix. The possibility of a superparamagnetic iron(III) doublet produced by very fine-grained magnetite or haematite has been tentatively ruled out by observing the low temperature (125 K) Mössbauer spectra of these samples. Preliminary transmission electron microscopy (TEM) studies also indicate the lack of a second phase. X-ray diffraction studies done by the Chinese on ferrifayalite indicate a single phase with a superstructure based on a vacancy-ordered olivine lattice^{8,9}.

Electron microprobe analyses (Table 3) of iron(III)-rich fayalites indicate ordinary fayalite with 3–4% Mn in the case of Mourne Mountains, Pantelleria, and St Peter's Dome, and about 1.5% Mg in Qianan ferrifayalite. Oxides summed to over 99% for Pantelleria and St Peter's Dome, but only 96% for Mourne Mountains and 92.5% for Qianan, a possible indicator of nonstoichiometry.

Table 1 Rock types in which fayalites of interest to this study were found

Locality	Description
Rockport, Massachusetts	Granite
Qianan Co., China	Early Archaean granulites and eulysites
Mourne Mts, Ireland	Granite
Pantelleria, Italy	Pantellerite pumice
St Peter's Dome, Colorado	Granite pegmatite

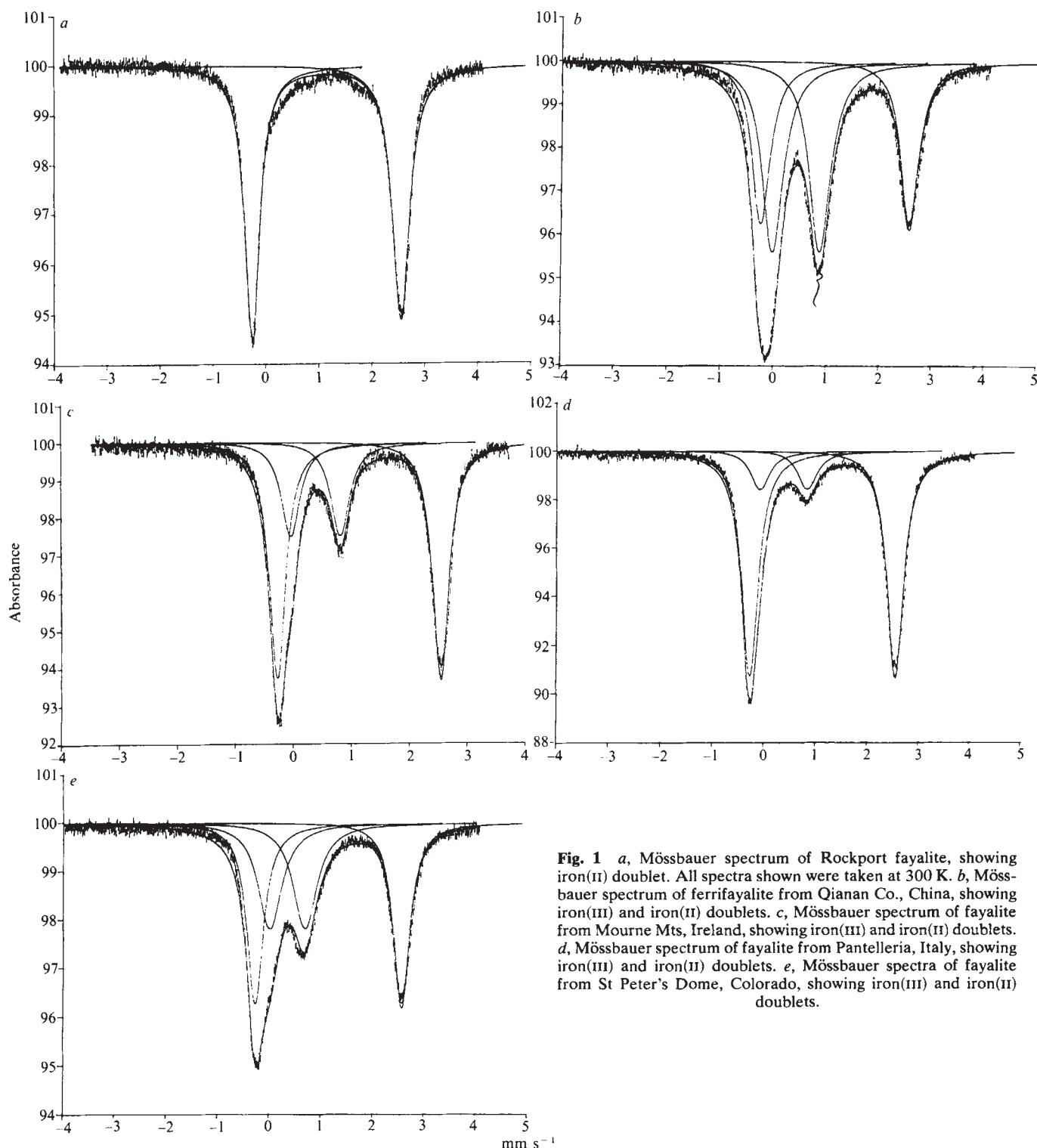


Fig. 1 *a*, Mössbauer spectrum of Rockport fayalite, showing iron(II) doublet. All spectra shown were taken at 300 K. *b*, Mössbauer spectrum of ferrifayalite from Qianan Co., China, showing iron(III) and iron(II) doublets. *c*, Mössbauer spectrum of fayalite from Mourne Mts, Ireland, showing iron(III) and iron(II) doublets. *d*, Mössbauer spectrum of fayalite from Pantelleria, Italy, showing iron(III) and iron(II) doublets. *e*, Mössbauer spectra of fayalite from St Peter's Dome, Colorado, showing iron(III) and iron(II) doublets.

A significant proportion of the fayalites examined from museum collections showed evidence of iron(III) in their structure. This high proportion of iron(III)-rich fayalites which may be lying unsuspected in museum collections brings to mind several interesting questions. Are these samples (disregarding the Chinese ferrifayalite for now) true fayalites, or are they misidentified examples of a new mineral, perhaps samples of the mineral ferrifayalite?

If these minerals are true fayalites, we must revise our definition of fayalite. It would not be a single phase, but a solid solution of iron(II) endmember fayalite and an iron(III) rich component. If so, its thermodynamic activity would not be unity, but would be <1 , and calculations using an activity of 1 must be reexamined. Its stability field would probably be

affected also, and care must be taken when performing experiments in the FeO-SiO₂ system to control or at least measure carefully the oxygen fugacity in the system.

If these iron(III)-bearing olivines are not true fayalites, but examples of a new mineral, I would suggest that existing fayalites in museums and private collections be examined carefully, perhaps analysed by X-ray diffraction or Mössbauer spectrometry to determine whether they have been misidentified. Also, studies of the possible formation conditions of this mineral should be of interest, since it must have a place in the FeO-SiO₂ system.

A third possibility for the classification of these minerals is merely a combination of the two previously mentioned. As fayalite with normal iron(II) and fayalite crystal structure

Table 2 Summary of Mössbauer parameters and iron(III)/(II) ratios of fayalites

Locality	Iron(II) peaks			Iron(III) peaks			Ratio of iron(III)/(II) peak areas
	Centre shift	Quadruple splitting	Peak widths	Centre shift	Quadruple splitting	Peak widths	
Rockport, Massachusetts	1.1667	2.8077	0.3442 0.3927				0
Qianan Co., China (ferrifayalite)	1.1687	2.8129	0.4161	0.4312	0.8893	0.4837	1.38
Mourne Mts, Ireland	1.1573	2.8164	0.3386	0.4071	0.8523	0.3877	0.46
Pantelleria, Italy	1.1541	2.8202	0.3872	0.3843	0.9123	0.4360	0.19
St Peter's Dome, Colorado	1.1657	2.8349	0.3885	0.3740	0.6785	0.5543	0.85
Artificial (from slag)	1.1656	2.8240	0.4453 0.4828				0
Dalarne, Sweden	1.1507	2.7910	0.4028 0.4466				0
Varmland, Sweden	1.1694	2.7987	0.2959 0.3597				0
Palmer Peninsula, Antarctica	1.1582	2.8233	0.3231 0.3795				0

Spectra containing four peaks were constrained such that areas and widths of peak pairs were equal, since there was extensive overlap of peaks. This was not necessary for two-peak spectra, and widths of both peaks are given for these. An iron-foil standard was used for all spectra.

Table 3 Microprobe analyses of fayalites of interest to this study

Oxides	Rockport	Qianan	Mourne Mts	Pantelleria	St Peter's Dome
SiO ₂	29.06 ± 0.15	30.97 ± 0.67	30.43 ± 0.19	29.72 ± 0.21	29.15 ± 0.39
Al ₂ O ₃	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
FeO	67.28 ± 0.33	59.97 ± 0.38	61.86 ± 0.36	63.48 ± 0.21	65.79 ± 0.27
MgO	0.08 ± 0.03	1.45 ± 0.03	0.38 ± 0.02	1.60 ± 0.04	0.00 ± 0.03
MnO	2.28 ± 0.07	0.08 ± 0.04	3.14 ± 0.09	4.08 ± 0.09	4.09 ± 0.13
TiO ₂	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.02	0.00 ± 0.03
CaO	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.02	0.51 ± 0.08	0.00 ± 0.00
K ₂ O	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total	98.71	96.06	95.89	99.40	99.04

All numbers are in wt %. FeO indicates total iron analysed as FeO.

exchanges more and more of its iron(II) for iron(III), it may at some point distort into the ferrifayalite (laihunite) crystal structure. At this point we would call it ferrifayalite. But chemically, one can still think of these two minerals as forming a solid solution, with fayalite as one endmember and ferrifayalite as the other, even though the crystal structure of the two endmembers is not identical. This classification of iron(III)-rich fayalites may be the most reasonable. It carries with it the implications mentioned for both of the other possibilities.

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Diagenesis of magnetic minerals in Recent haemipelagic sediments

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Rapidly deposited sediments from marine and lake environments are being used increasingly to study decadal to millennial fluctuations in the Earth's magnetic field. The objectives are to gain more fundamental understanding of the geodynamo and to establish a new dating technique for sediments. While lacustrine sections are generally restricted to temperate latitude glacial lakes ($\leq 20,000$ yr old), rapidly deposited marine sediments along continental margins potentially offer continuous high resolution, yet long-term records of geomagnetic secular variation. However, to interpret the sedimentary magnetic record accurately, geochemical processes that affect the reliability of the magnetic signal must be understood. We now report downcore magnetic profiles from undisturbed Kasten cores taken in rapidly deposited laminated sediments from the Gulf of California and in bioturbated haemipelagic muds on the Oregon continental slope which give apparently reliable directions, but show dramatic decreases in the intensities of natural (NRM) and artificial (ARM, IRM) remanences with depth.

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Downcore porewater and solid sulphur analyses show concave-down decreases in porewater sulphate and systematic increases in pyrite and metastable monosulphides. The maximum curvature of the sulphide profile occurs directly below the high magnetization zone. Combined with other compositional and mineralogical analyses, these data suggest that due to oxidative decomposition of organic matter, magnetites and other iron oxides become progressively reduced and subsequently sulphidized and pyritized with depth. Iron reduction seems to occur before sulphide formation. Changes in magnetic stability parameters are consistent with selective dissolution of the finer-sized grains causing downcore coarsening of the magnetic fraction.

The ultimate cause of early diagenesis in marine sediments is the decomposition of organic matter by microbial oxidation. The rates of decomposition and effects on the substrate depend mainly on the availability and reactivity of both organic matter and reductants and the competitive efficiency of microbial populations. Microbes tend to reduce those chemical species which produce the most energy. Thus, in porewaters, reduction tends to occur according to a well defined sequence determined by the free energies of the reactions^{1,2}. In the porewaters of suboxic sediments, Froelich *et al.*³ found a systematic succession of downcore diagenetic reactions proceeding from direct oxidation (by O_2) to manganese reduction, nitrate reduction and ammonia formation. After consumption of labile MnO_2 and NO_3^- , iron oxides are reduced, imparting the characteristic olive grey-green colour to many haemipelagic muds. If sufficient metabolizable organic matter is still present, sulphate reduction begins, followed eventually by fermentation and concomitant methane production. At depths where sulphate is reduced, iron (II) in the porewaters becomes sulphidized, first forming metastable monosulphides (for example, mackinawite) and subsequently pyrite⁴⁻⁷.

In reducing environments, well crystallized iron oxides have usually been considered to be relatively inert⁸. However, recent recalculations of phase equilibria in the Fe-S-H₂O system⁹ suggest that the common ferrimagnetic oxides (magnetite, haematite and maghaemite) may not be thermodynamically stable in the E_h -pH conditions found in the porewaters of some anoxic sediments.

Oceanic regimes which are amenable to high-resolution palaeomagnetic studies are those having high terrigenous sedimentation rates. These areas, generally adjacent to continental margins, also contain large amounts of organic matter,

in most cases because of increased fluviially derived terrigenous carbon input, upwelling-induced high oceanic carbon input, and enhanced preservation due to rapid burial¹⁰. As a consequence of the high carbon input, direct oxidation (by O_2), if present, is confined to the upper few centimetres of sediment, although this layer generally thickens going offshore². If sufficient organic matter is present, the remainder of the sediment column is anoxic and also sulphidic, because sulphate is abundant in seawater. This two-layer model for haemipelagic sediments contrasts with the deep-sea pelagic 'red' clay regime, where due to low organic input and slow sedimentation, oxidizing conditions can prevail throughout the entire sediment column¹¹.

We have made a detailed comparative study of the magnetic, geochemical and sedimentological properties of sediments from two contrasting depositional environments, the Gulf of California and the Oregon continental margin. BAM-80-E17 ($\phi 27^\circ 35.2' N$, $\lambda 111^\circ 36.6' W$, depth = 625 m) is a 4.5-m Kasten core taken in the Guaymas Basin where the O_2 minimum intersects the slope. The sediments are composed of undisturbed, finely laminated couplets of light coloured diatom-rich ooze alternating with darker organic-terrigenous-diatomaceous muds. Varve chronologies and three ^{14}C dates give sedimentation rates between 140 and 160 cm kyr⁻¹. Major element chemistry shows high correlations ($r > 0.9$) between the terrigenous elements Al, K, Fe and Ti, suggesting downcore mixing of a single terrigenous assemblage, such as from the nearby Rio Yaqui, with Si-rich material, presumably diatoms. This simple model is also supported by X-ray mineralogy and Mössbauer studies.

Downcore magnetic profiles with replicated sampling give stable and apparently reliable directions. The core mean inclination of 41.1° ($\alpha_{95} = 2^\circ$) is comparable to the expected geocentric axial dipole (EGAD) inclination of 46.3° . However, the striking feature of downcore NRM and laboratory-produced anhysteretic (ARM) remanent magnetization profiles, partially demagnetized at 100 Oe a.f. (Fig. 1a), is the precipitous decrease in magnetic intensity by over an order of magnitude from the surface to 20–30 cm, whereupon intensities become relatively constant. The stabilities of NRM and ARM, as measured by the median demagnetizing field (MDF), also show a steplike decrease at this depth (Fig. 1b).

X-ray powder diffraction patterns of magnetic mineral separates indicate that the top 30 cm consist mainly of pure magnetite ($a_0 = 8.39 \text{ \AA}$) with minor amounts of haematite. Ferrimagnetic

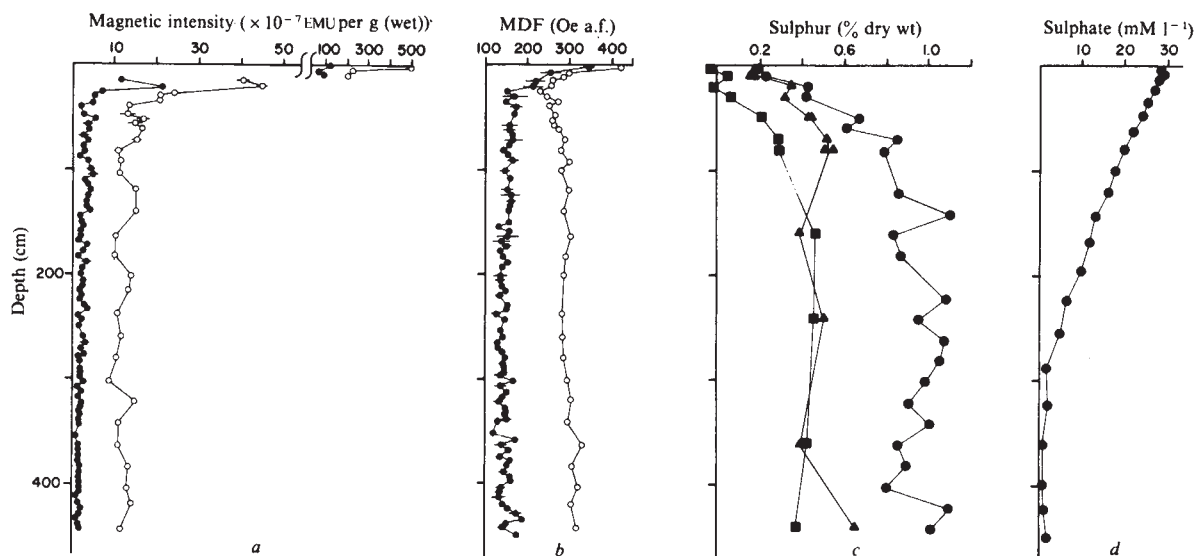
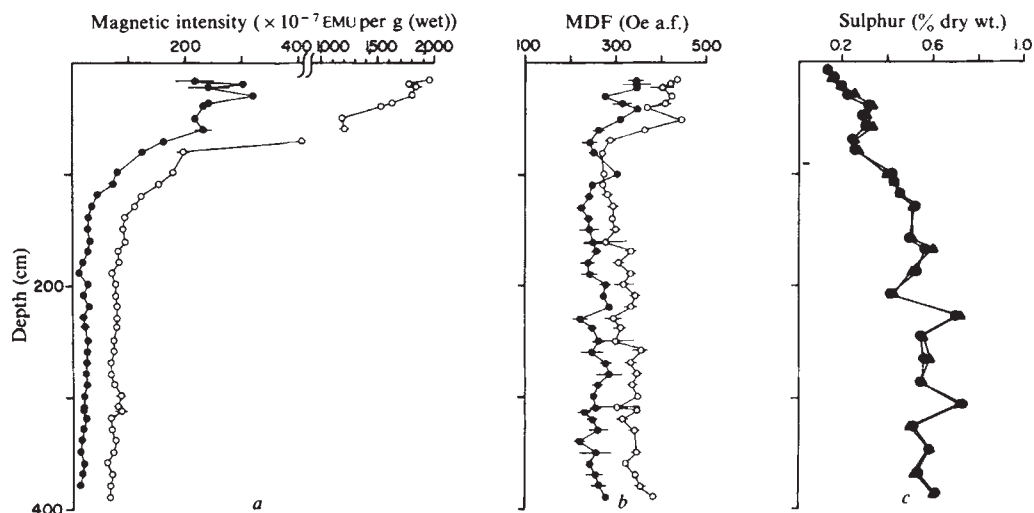


Fig. 1 Downcore magnetic properties and sulphur profiles of Kasten core BAM 80-E17, Guaymas Basin, Gulf of California. *a*, Magnetization intensities, partially demagnetized at 100 Oe a.f.; *b*, median demagnetizing fields of NRM (●) and ARM (○); *c*, sulphate-corrected solid sulphur concentrations of total (●), acid-insoluble (▲) and acid-volatile (■) fractions; *d*, porewater sulphate in mmol l⁻¹. Total sulphur was determined by X-ray fluorescence and LECO analyser; acid-insoluble sulphur was measured by LECO. Acid-volatile is total minus acid-insoluble sulphur, as obtained from LECO.

Fig. 2 Downcore magnetic properties and sulphur profiles of Kasten core W7710-28, Oregon continental slope. *a*, Magnetization intensities, partially demagnetized at 150 Oe a.f.; *b*, median demagnetizing fields of NRM (●) and ARM (○); *c*, solid sulphur concentrations of total (●) and acid-insoluble (▲) fractions on a sulphate-free basis, as measured by X-ray fluorescence.



griegite (Fe_3S_4) was not observed. Below 30 cm, the only magnetic species present in the concentrates is pure magnetite. Isothermal remanence acquisition curves¹², as well as the X-ray results and ARM stabilities¹³, suggest that the remanence throughout the core is controlled by fine-grained magnetite.

Core W7710-28 is a 3.9-m Kasten core ($\phi 44^\circ 50.1' \text{N}$, $\lambda 125^\circ 07.5' \text{W}$ water depth: 1,825 m) taken in a small depositional basin on the lower Oregon continental slope. The sediment consists of heavily bioturbated, mottled to homogeneous olive-green haemipelagic mud. The common red-brown surficial oxic layer was not observed, and a 30 cm boxcore at the same site contained numerous worms, tubules and burrows. Correlation with another ^{14}C -dated core at the same site indicates that the sedimentation rate is $\sim 125 \text{ cm kyr}^{-1}$.

Downcore X-ray determinations and chemical analyses suggest complex mineralogical assemblages which show no coherent variations with sub-bottom depth. Mössbauer studies of bulk material show a remarkable downcore constancy in the chemical properties of the dominant iron-rich species (mainly detrital phyllosilicates).

Magnetic directions are stable and replicable within horizons. The core mean inclination of 62° ($\alpha_{95} = 2^\circ$) closely approximates the EGAD inclination of 63° . However, like the Guaymas Basin sediments, these muds also show a strong downcore decrease in NRM and ARM intensities ('cleaned' at 150 Oe a.f.) and a decrease in MDF with depth (Fig. 2*a, b*), with the maximum change occurring between 70 and 100 cm. Magnetic concentrates contain only magnetite ($a_0 = 8.39 \text{ \AA}$) throughout the core. The NRM and ARM stabilities (MDFs $> 200 \text{ Oe}$) suggest that the magnetite is submicrometre in size¹³.

Analyses of sedimentary sulphur in the Guaymas and Oregon sediments (Fig. 1*c, 2c*) show an inverse relation between sulphur and magnetic intensity. For the Guaymas sediments, profiles of sulphate-free fractions of total, acid-insoluble in 0.1 M HCl (pyrite + organic sulphur), and acid-volatile (metastable monosulphides) sulphur show systematic increases downcore, reaching relatively constant values at 100–150 cm. The maximum rates of increase occur directly below the zone of high magnetization. In contrast, the porewater sulphate profile (H. Brumsack, personal communication) shows an exponential downcore decrease, indicating sulphate reduction to a depth of $\sim 300 \text{ cm}$ (Fig. 1*d*). Downcore sulphur profiles for the Oregon suboxic muds show analogous trends. Acid-insoluble sulphur, presumably in pyrite, and total sulphur increase downcore, reaching relatively constant values at 110–130 cm, directly beneath the zone of high magnetization. An appreciable acid-volatile fraction is not present.

In view of the models of iron and sulphur diagenesis described earlier, these diverse analyses yield a consistent picture of iron diagenesis. In the top 20–30 cm of the anoxic muds of the Guaymas Basin, the iron in fine-grained magnetic oxides (as

well as other iron minerals) becomes reduced by reaction with organic matter. Changes in NRM and ARM intensities and stabilities are consistent with selective dissolution of the smaller grains, leading to downcore coarsening of the magnetic fraction.

Iron reduction must occur separately from and before sulphide formation as intermediate metastable sulphides are not produced in the top 20–30 cm, and the various sulphide maxima occur at 50–100 cm, well below the iron reduction zone. This conclusion is in accord with laboratory experiments which have found that iron reduction is independent of sulphate reduction and that nitrate-reducing rather than sulphate-reducing bacteria seem to be responsible for iron reduction¹⁴. Thus, reduced iron must either diffuse downwards or form an unstable iron (II) oxyhydroxide, silicate or iron-organic chelate which is eventually moved into the zone of sulphidization and pyritization. The iron (II) then reacts with hydrogen sulphide, derived from sulphate reduction, to form mackinawite (FeS) which inverts to pyrite on reaction with elemental sulphur. Because the concentration profiles of the sulphide species are not mirror images of the porewater sulphate profile, the rates of monosulphide formation and pyritization must be controlled by the availability of reactive iron rather than of sulphide.

Similar processes can be recognized in the sub-oxic to anoxic Oregon muds, except that iron reduction and pyritization begins deeper in the sediments, probably as a result of the more oxidizing conditions in the water column and bioturbation in the surficial sediments.

The reduction and dissolution of iron oxides is important to palaeomagnetists studying rapidly deposited sediments in both lacustrine and marine environments. Because iron reduction is not directly coupled to sulphide formation, iron oxides in lake sediments might also undergo reduction with accompanying decreases in remanence and other changes in magnetic properties with depth. Rather than forming sulphides, iron (II) may accumulate to appreciable levels in the porewaters of lakes, eventually reacting to form authigenic minerals, such as siderite (FeCO_3) or vivianite ($\text{Fe}(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$) (ref. 15).

Although iron reduction seems to have little effect on the reliability of palaeomagnetic directions (R.K. and S.L., in preparation), questions arise as to the validity of relative palaeointensity determinations in diagenetically altered sediments. Such estimates rely on the assumptions that the distribution of remanence carriers is uniform throughout the section and that magnetic mineral concentration and field are the only independent variables responsible for the primary remanence¹⁶. Unless (1) NRM stabilities remain constant downcore, and (2) concentration normalizing parameters (for example, ARM) respond to dissolution-induced grain size changes in an identical manner to NRM, the above assumption(s) will be violated and relative palaeointensity determinations will give erroneous estimates of palaeofield behaviour.

In both oceanic and lacustrine sediments, the extent of iron diagenesis and consequent effects on magnetic properties will depend on the degree to which reducing conditions are established, as well as the initial grain size distribution and crystallinity of the magnetic species. As the redox potential is primarily controlled by the organic matter accumulation rate, the effects of iron reduction will be magnified in regions such as continental borderlands where sedimentation rates are high and the magnetic fraction is fine-grained.

Downcore variations in magnetic properties (such as NRM, ARM, IRM or χ , low field susceptibility) may provide rapid, nondestructive diagnostic tools for geochemists in identifying zones where iron reduction is occurring. In the sedimentary record, where changing climate and oceanic parameters may induce fluctuating redox conditions in the surficial sediments, variations in pyrite concentrations, or conversely, drastic changes in remanence intensity and stability may provide fingerprints of ancient geochemical boundaries. The recognition of these diagenetic processes may become increasingly important as the Deep Sea Drilling Project's Hydraulic Piston Corer provides more long undisturbed sedimentary sections for palaeomagnetic study.

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A mammal-like reptile from Australia

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New fossil evidence indicates that a mammal-like reptile inhabited Australia in the early part of the Triassic period (~220 Myr ago). There are no previous reports of mammal-like reptiles from this continent, and the earliest known Australian mammals have been dated as no older than Oligocene (~23 Myr)^{1,2}. The evidence is an isolated quadrate bone, recently discovered in Lower Triassic rocks of the Arcadia Formation, south-east Queensland. This bone has morphological peculiarities matched only in dicynodonts (mammal-like reptiles of the infraorder Dicynodontia, order Therapsida) and was probably derived from an animal similar or identical to the common African dicynodont *Kannemeyeria*.

The specimen (Fig. 1a, b) was collected from freshwater sediments of the Arcadia Formation at a locality known as the Crater, approximately 72 km south-west of Rolleston, south-east Queensland (field locality L78 in Queensland Museum records). The Arcadia Formation (formerly named the Rewan

Formation³) has already yielded a rich vertebrate fauna⁴ including diverse labyrinthodont amphibians^{5–8}, a thecodontian related to *Chasmatosaurus*, an eosuchian similar to *Pro-lacerta*¹⁰, a paliguanid¹⁰, a proclophionid, a dipnoan, and the subholostean *Saurichthys*¹¹. This fauna has affinities with that of the South African *Lystrosaurus* Zone (Lower Triassic) and its equivalents in other Gondwana continents^{7,9}. Palynological evidence¹² also supports correlation of the Arcadia Formation with the *Lystrosaurus* Zone.

The newly discovered quadrate is an unusually short, thick and very robust bone, with two strongly developed condyles separated by a deep V-shaped fossa. Its medial condyle is preserved almost intact and is highly distinctive in shape; it resembles part of the rim of a thick wheel, extending through an arc of ~150°. The lateral condyle is damaged but was considerably larger; it seems to have been broadly convex in form (both transversely and longitudinally) and certainly extended a little more ventrally than the medial condyle. The intercondylar fossa is aligned longitudinally, so that the entire articular surface of the quadrate is pulley-like rather than helical. This particular pattern of condyle morphology appears to be unique to dicynodonts¹³.

The specimen finds its closest match in the dicynodont *Kannemeyeria*^{13–16}, being virtually identical to one example illustrated by Watson¹³ (Fig. 1c, d). In view of this extremely close resemblance, the Arcadia quadrate is provisionally identified as that of a dicynodont closely related (or perhaps even identical) to *Kannemeyeria*. The genus *Kannemeyeria* is best known from the Lower Triassic (*Cynognathus* Zone) of South Africa^{14,15,17}, but has also been reported in the Manda Formation of Tanzania^{17,18}, the N'tawere Formation of

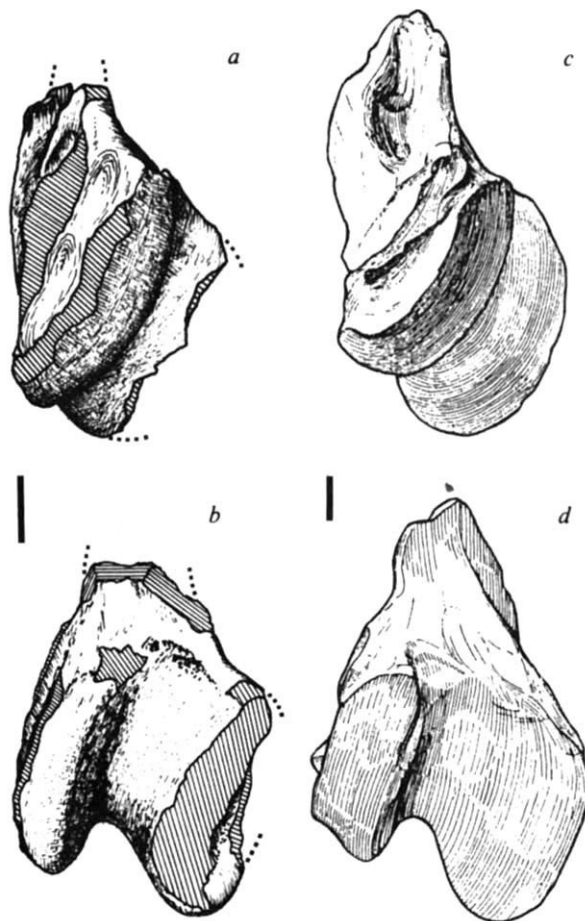


Fig. 1 Left quadrate of dicynodont reptile from the Arcadia Formation, Queensland (a, b, Queensland Museum F 12178), compared to that of an African specimen of the dicynodont *Kannemeyeria* (c, d, British Museum (Natural History) R 3763, from Watson¹³). a and c are medial views; b and d are anterior views. Scale bars, 1 cm.

Zambia^{17,19}, the Omingonde Formation of Namibia²⁰, the Puesto Viejo Formation of Argentina¹⁶, and the Yerrapalli Formation of India^{17,21}; closely related genera are present in the Er-ma-Ying Formation of China²². The earliest of these occurrences is probably in the Puesto Viejo Formation, where *Kannemeyeria* may have been contemporary with *Lystrosaurus*; the latest occurrence is probably in the Manda Formation (Middle Triassic, Anisian). If the quadrate described here does represent *Kannemeyeria* (or a closely related dicynodont) its occurrence would confirm that the Arcadia fauna is of Triassic (and probably Lower Triassic) age—though it would not necessarily support a correlation with the *Lystrosaurus* Zone.

Dicynodonts were among the most successful of all mammal-like reptiles; they are known to have ranged from the late Permian into the late Triassic²³, and they have been reported hitherto from every continent except Australia. In some environments dicynodonts were extremely abundant; for example, Colbert estimated²⁴ that about 85% of the vertebrates recovered from the *Lystrosaurus* Zone of South Africa were specimens of the single dicynodont genus *Lystrosaurus*. This same genus is also known, and sometimes quite commonly, from South America, Russia, India, China and Antarctica. In the *Cynognathus* Zone of South Africa the abundance of *Kannemeyeria* prompted Cruickshank to comment²⁵ that this animal was perhaps a better zone fossil than *Cynognathus*.

It now appears that Australia also supported a dicynodont fauna similar to that in other Gondwana continents, but that the fossil evidence remains largely undiscovered. Here it must be borne in mind that suitably fossiliferous exposures of Triassic rocks are very scarce in Australia. For example, there are, to my knowledge, only four productive exposures of the Arcadia Formation—and three of these have yielded little more than rare scraps of bone. It may also be noted that the mammal-like reptiles of the other Gondwana continents sometimes occur in local concentrations^{26,27}; this mode of occurrence would certainly reduce the likelihood of discovering the animals, especially when exposures are so few.

It seems certain that dicynodonts were not involved in the ancestry of mammals^{23,28}. Consequently the discovery of a dicynodont in the Arcadia Formation can have no direct bearing on the origin of Australia's unique mammal fauna. Nevertheless there is one fact that does raise some interesting possibilities: in all the other Gondwana continents dicynodonts are found in association with cynodonts (therapsid suborder Cynodontia)—which probably do include the ancestors of mammals. It is therefore possible that cynodonts were also present in Australia.

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New fossil discoveries from the Miocene of Nepal include a hominoid

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We present here preliminary findings of recent field studies on Miocene Lower Siwaliks deposits of southwestern Nepal. Twenty-four vertebrate taxa are now represented from over 50 localities. The fauna is largely aquatic, being dominated by fish, turtles, crocodiles and aquatic snakes. Lithologies indicate deposition in sluggish streams and associated environments. Biostratigraphy indicates correlation between the composite Nepal fauna and Lower Siwaliks faunas of India and Pakistan, a conclusion corroborated by a palaeomagnetic study showing an age of 9–10 Myr for some of the fossil sites. A hominoid specimen, found in Tinau Khola, is 9.0–9.5-Myr old. It is similar to several specimens of small Miocene hominoids from Pakistan and India, and helps fill the geographical gap between hominoids collected previously in northwestern India and southern China.

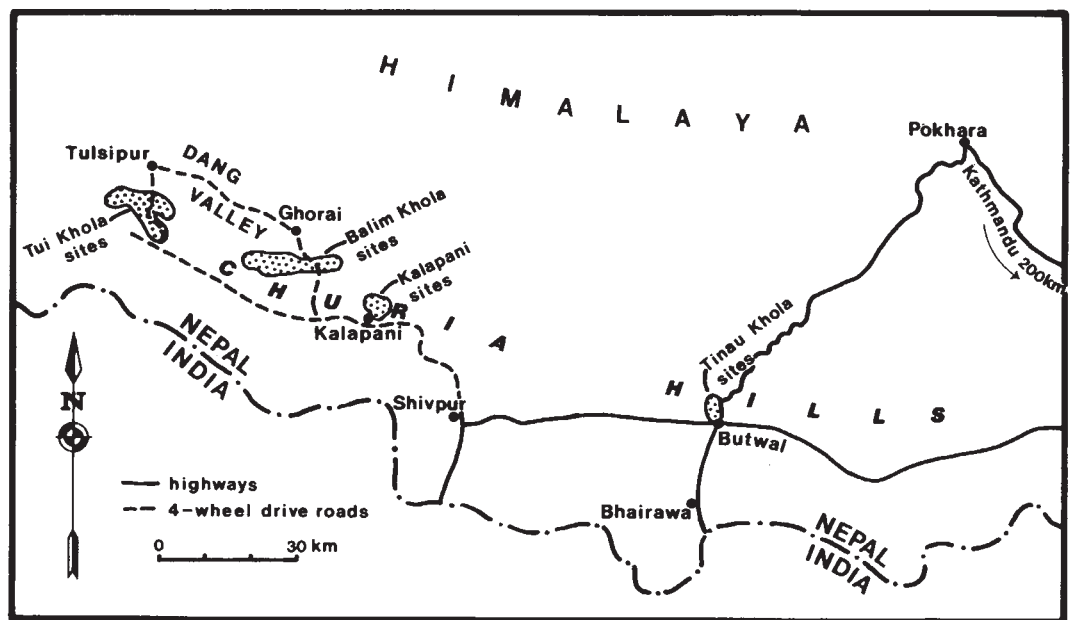
The Siwalik Group is a thick fluvial sedimentary sequence deposited during middle Miocene through middle Pleistocene time along the southern flanks of the Himalaya. These rocks have been studied palaeontologically in India and Pakistan for almost 150 yr, but it is only recently that such studies have extended into Nepal. The first fossil collection of any size from Nepal, consisting of 52 vertebrate specimens from 17 localities, was made in 1976 in the Dang Valley¹. Subsequent aerial photography of this and other areas of western Nepal pinpointed rock exposures likely to yield more vertebrate fossils.

Field work conducted between 1979 and 1982 has substantially increased our knowledge of Nepal's Miocene vertebrate fauna. A few specimens, including representatives of the equid *Hipparion*, have been collected from the Middle Siwaliks. Most, however, are from the Lower Siwaliks. Twenty-four vertebrate taxa (Table 1) are known from over 50 Lower Siwaliks sites (Fig. 1).

Lower Siwaliks vertebrate fossils are generally found on the surface, having weathered out of siltstone to fine-grained sandstone, sandy limestone, and marlstone. This range of lithologies contrasts rather strongly with the Lower Siwaliks in India and Pakistan, where sandstone and siltstone are the dominant lithologies and carbonates are rare. This makes lithologic correlation difficult and suggests that the Nepal Lower Siwaliks were deposited in poorly drained areas with sluggish streams, sloughs, and ponds.

Low density of fossils in Nepal, relative to many Lower Siwaliks sites in India and Pakistan, may be related to these apparently different environments of deposition. The rarity of

Fig. 1 Part of south-western Nepal showing areas of Lower Siwaliks studied by 1976–82 expeditions. The hominoid specimen was collected at Tinau Khola.



fossils has thus far prevented successful excavation for large specimens and screen-washing for small-vertebrate specimens.

Faunal composition reflects aquatic environments of deposition and is probably representative of proximal communities: 34% of the vertebrate specimens from Nepal are fish; turtles, crocodiles and snakes account for 43% of the specimens; only 23% are mammals.

Just as different lithologies make lithostratigraphical correlation of the Siwaliks from Nepal to India difficult, the largely aquatic Nepal Lower Siwaliks fauna is difficult to correlate biostratigraphically with the taxonomically diverse but dominantly terrestrial pre-*Hipparion* faunas of India and Pakistan. Several Nepal fossil mammals, however, are biostratigraphically useful. The boselaphines *Protragocerus gluten* and *Sivoreas eremita* are characteristic of the 'Chinji fauna' of the Indopakistan Lower Siwaliks (Alan Gentry, personal communication). *Conohyus sindiensis* became extinct in Pakistan about 9.5 Myr ago², and the single rhizomyid incisor from Tinau Khola is of the small size typical of 10-Myr old representatives

of this family in Pakistan. Our preliminary conclusion is that the composite Nepal Lower Siwaliks fauna correlates with Lower Siwaliks faunas of Pakistan and probably is older than 9 Myr. However, note that only the most recent studies in Pakistan²⁻⁴ have convincingly correlated year dates with biostratigraphical events in the Siwaliks.

One of the most important fossils yet collected in Nepal is a hominoid tooth from Tinau Khola. It was found *in situ* at the top of a gray mudstone bed on the west bank of Tinau Khola (27°44'N, 83°28'E), ~2 km north of Butwal, Lumbini Zone, central southern Nepal (Fig. 1).

The hominoid specimen (Fig. 2) is a well-preserved left upper first molar 10.0 mm long and 10.9 mm wide. The crown is complete except for minor distolingual enamel spalling, but the roots are not preserved. The occlusal outline is rhomboidal. Prominent interdental wear facets are present at the centre of the mesial surface and across approximately half the distal surface. The occlusal surface is moderately worn, with sharply defined wear facets. The enamel is very thick; no dentine is exposed. The protocone is the largest cusp, the hypocone smallest, and the paracone and metacone are of approximately equal size. A small mesial fovea is terminated lingually by a worn protoconule. The occlusal and distal foveae are approximately equal in size. Crenulation of the enamel is particularly well-developed in the distal fovea.

Several Asian small-hominoid specimens include first upper molars which are morphologically indistinguishable from the Tinau Khola specimen, aside from 10–15% variation in length and width measurements. Among these are GSP (Geological Survey of Pakistan) 5019⁵ from the Potwar area of Pakistan, K56 675 (Geological Survey of India) from Ramnagar and YPM (Yale Peabody Museum) 13799⁶, the type of *Ramapithecus brevirostris* (*R. punjabicus*)⁷ from Haritalyangar. The latter two specimens are from northwestern India.

These small Siwaliks hominoids traditionally have been referred to *Ramapithecus punjabicus*. However, recent work⁸⁻¹⁰ suggests that the names *Sivapithecus* and *Ramapithecus* have been applied to a single genus. *Sivapithecus* is the earlier valid name for this taxon, and all small Siwaliks hominoids probably represent a single species. We follow Andrews¹⁰ in applying the name *Sivapithecus punjabicus* to this species and to the Tinau Khola specimen. Pilbeam⁹ presents evidence suggesting that *Sivapithecus* is a pongid; we therefore place the Nepal hominoid in the Pongidae.

The age of the Tinau Khola hominoid specimen has been determined primarily palaeomagnetically. *Conohyus sindiensis* and a rhizomyid are found in Tinau Khola in close stratigraphic

Table 1 Vertebrate taxa from Nepal Lower Siwaliks

Osteichthyes	Mammalia
Ophiocephaloformes	Creodonta
Channidae	Hyaenodontidae
<i>Channa</i> sp.	Carnivora
Cypriniformes	Amphicyonidae
cf. Cyprinidae	<i>Amphicyon palaeindicus</i>
Siluriformes	Proboscidea
Clariidae	Gomphotheriidae
	Deinotheriidae
	<i>Deinotherium pentapotamidae</i>
Reptilia	Perissodactyla
Testudines	Rhinocerotidae
Trionychidae	<i>Brachypotherium perimense</i>
<i>Lissimys punctata</i>	Artiodactyla
<i>Trionyx/Chitra</i> sp.	Suidae
Testudinidae	* <i>Conohyus sindiensis</i>
<i>Geochelone</i>	Anthracotheriidae
Emyidae	<i>Hemimeryx pusillus</i>
<i>Batagur</i>	Tragulidae
* <i>Emyids</i> , 2 spp., indet.	<i>Dorcatherium</i> sp.
Squamata	Bovidae
Boidae	<i>Sivoreas eremita</i>
<i>Acrochordus dehmi</i>	<i>Protragocerus gluten</i>
	cf. <i>Pachyporax</i> sp.
Crocodylia	Rodentia
Crocodylidae	*Rhizomyidae
* <i>Crocodylus</i> sp.	Primates
Gavialidae	Pongidae
<i>Gavialis</i> sp.	* <i>Sivapithecus punjabicus</i>

* Taxa associated with hominoid at Tinau Khola.

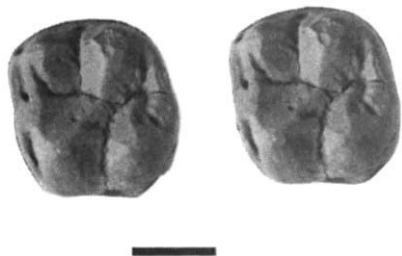


Fig. 2 Stereo photographs of hominoid left upper first molar, from Lower Siwaliks of Tinnau Khola. Scale bar, 5 mm.

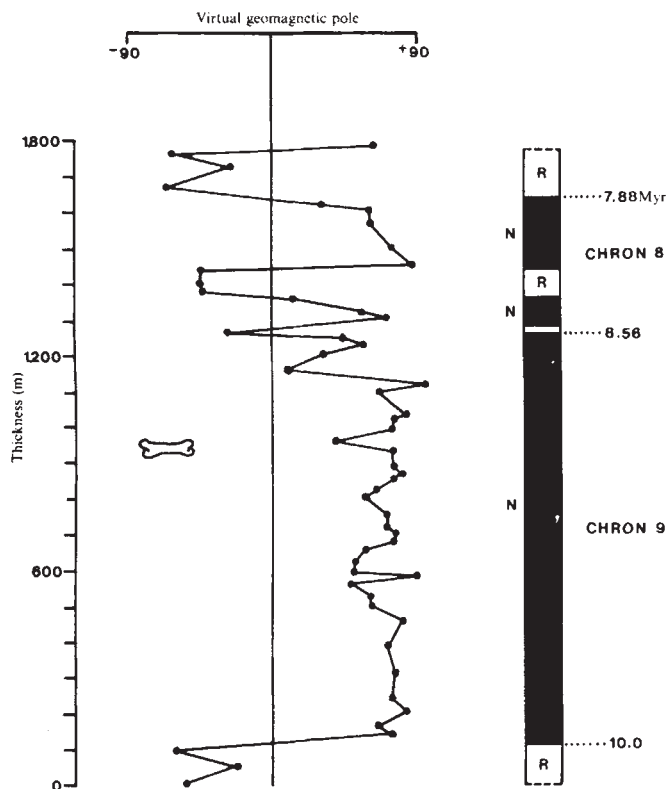


Fig. 3 Magnetic polarity stratigraphy of Tinnau Khola section. Fossiliferous zone indicated by bone symbol. Radiometric calibration from ref. 12. Magnetic reversals correlated to ref. 11.

proximity to the hominoid, and they suggest an age around 10 Myr as noted above, but greater precision is provided by the palaeomagnetic evidence.

The fossiliferous zone at Tinnau Khola is ~800–900 m above the base of a steeply dipping section which is continuously exposed over a stratigraphical interval of approximately 1,800 m. This section consists of cyclically alternating sandstone, marlstone, and mudstone beds with occasional thin conglomerate beds. A total of 126 samples from 63 sites spanning this 1,800-m section were analysed with a cryogenic magnetometer at the University of Wisconsin–Milwaukee (Fig. 3). One sample from each 100 m of section was demagnetized in detail using either alternating field demagnetization or thermal demagnetization. In some instances both alternating field and thermal demagnetization were performed on different specimens from the same hand-sample. Typically, alternating field demagnetization indicated a small secondary magnetization which could be removed by demagnetization to 300 Oe, after which the magnetization direction did not change when demagnetized up to 1,000 Oe. Thermal demagnetization showed similar results and generally magnetization was lost between 300 and 600 °C.

All remaining samples were demagnetized in alternating field to 300 Oe and the magnetic directions were rotated to remove

the regional dip of the sediments, which ranged between 30° and 55° to the north. After rotation the sample inclinations were typically between 5° and 30°, which is consistent for Nepal in the time range of interest.

The virtual geomagnetic pole latitudes are plotted in Fig. 3. Each point is an average for two hand-samples from the same level in the section. These results are consistent with results from an independent pilot set of 36 samples from 18 levels collected in 1981.

Results of the palaeomagnetic analysis of the Tinnau Khola section correlate well with previous Siwaliks palaeomagnetic studies¹¹ and indicate an age of 9.0–9.5 Myr for the Nepal hominoid. This is older than most dated hominoid specimens from the Siwaliks of Pakistan^{2–4}.

The Nepal specimen extends the known geographical range of Miocene hominoids some 400 km to the east-southeast of the nearest Indian site in the Kumaon Himalaya (Ashok Sahni, (personal communication). The easternmost Asian hominoid locality, in Yunnan Province in China^{13–15}, is ~1,500 km east of Tinnau Khola. The Nepal occurrence therefore helps complete the Asian distributional record of Miocene hominoids.

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Nitrate respiration in primitive eukaryotes

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Nitrate respiration plays an important part in the global nitrogen cycle by initiating a chain of biologically mediated reductions of nitrogen oxides^{1,2}. It involves the substitution of nitrate for oxygen as a terminal electron acceptor, occurs only in the absence of oxygen and is assumed to be performed only by prokaryotic organisms^{3,4}. Here we present evidence for nitrate respiration in eukaryotes. Some Protozoa are known to be capable of living in anoxic water in lakes^{5,6}; we show that in at least one genus of Protozoa (*Loxodes*) this capacity can be attributed to a dissimilatory nitrate reductase located within the inner mitochondrial membrane. The general acceptance of *Loxodes* as an extremely primitive protozoan⁷ strengthens the theory that nitrate-respiring bacteria are plausible ancestors of animal mitochondria^{8,9}.

Priest Pot is a small (1 hectare) water body in the English Lake District (54°22' N, 3°00' W). It is typical of many throughout the world in which the bottom water periodically becomes anoxic¹⁰. The protozoan biomass of the anoxic water

column is dominated by a community of large (length $> 150 \mu\text{m}$) ciliates which often reach extremely high densities (up to $8 \times 10^5 \text{ l}^{-1}$). Most of these ciliates belong to the primitive genus *Loxodes*. The anoxic water column is virtually devoid of other microfauna.

While examining the vertical distribution of these Protozoa with close-interval (10–25 cm) sampling it was discovered that

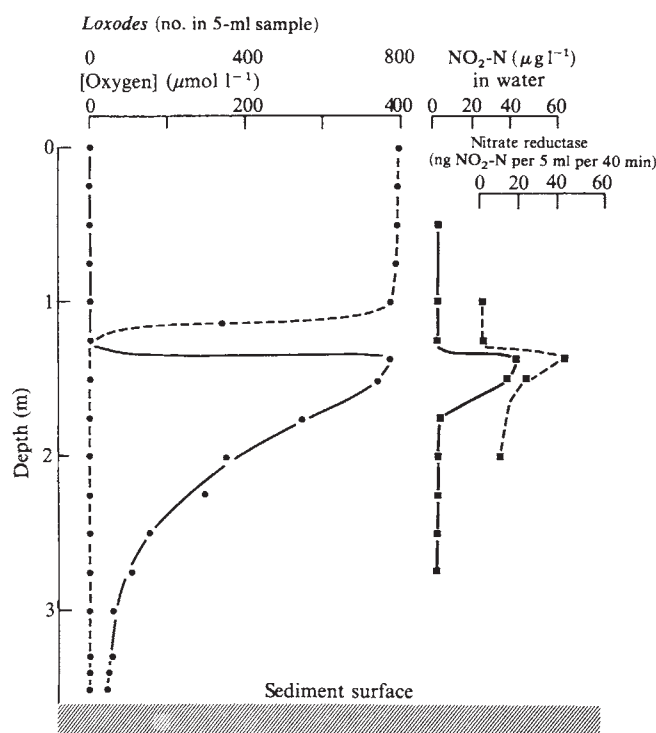


Fig. 1 Vertical distribution of *Loxodes* (●—●), dissolved oxygen (—●—●), nitrite (■—■) and nitrate reductase (■—■) in the water column of Priest Pot on 14 July 1982. Dissolved oxygen was determined using a YSI meter and stirrer-assisted electrode. The depth of the oxic-anoxic boundary was confirmed by polarographic measurements of oxygen in pump-sampled water²². A peristaltic pump was used to collect further samples at each depth²³. Samples (5 ml) were fixed with 0.6 ml 2% OsO_4 . The protozoa in these samples were concentrated by gentle centrifugation (90g for 60), transferred to glass Sedgewick-Rafter chambers and identified and counted. Nitrite was determined²⁴ in filtered (GF/C) water and nitrate reductase on the suspended material retained by 30- μm nylon sieves using the modified²⁵ method of Lowe and Evans²⁶. Enzyme activities were determined after incubation at 30°C for 40 min. The pond was 3.6 m deep on this occasion.

peaks in their abundance coincided with peaks of both nitrite concentration in the water and nitrate reductase activity in the fraction retained on a 30- μm sieve (Fig. 1). Similar coincident peaks were observed on over 20 occasions in two successive years. In every case these peaks were confined to anoxic water. This suggested that the sieve retentate, composed mainly of two species of *Loxodes*, contained a dissimilatory nitrate reductase (DNR) which was responsible for the associated accumulation of nitrite in the hypolimnion. Peaks of DNR activity would normally be attributed to nitrate-respiring bacteria but in this case the enzyme activity was retained on a sieve with 30- μm pores through which almost all unattached bacteria would pass. Bacteria attached to larger particles could have been retained but microscopic examination (light, scanning (SEM) and transmission (TEM) electron microscopy) of the most abundant of the available surfaces, the external surfaces of *Loxodes*, indicated that these were invariably free of bacterial contaminants.

Corroborative evidence for a DNR located in *Loxodes* was provided by a TEM investigation of thin sections of *Loxodes magnus*. Representatives of this species were isolated from aerobic sediment during April and May 1981 and from anoxic water in July and August. Randomly selected areas of sections were photographed and analysed using stereological techniques (Table 1). Mitochondria were retained in *L. magnus* living in anoxic water. Moreover, cells living anaerobically contained approximately 70% more mitochondria than those living in the presence of oxygen. This increase in number of mitochondria was accompanied by an increase in energy-yielding surface area—the area of cristae formed by infolding of the inner mitochondrial membrane. This increased area is, we suspect, a response to anoxia and a response induced by the lower efficiency of nitrate respiration over aerobic respiration. The evidence available for bacteria⁴ indicates that a reduction in molar growth yield of ~50% is associated with the switch from oxygen to nitrate respiration. The reduction in energy made available by the latter could be offset by increasing the number of shortened electron transport chains in the inner mitochondrial membrane. This would presumably require an increase in membrane surface area similar to that recorded here. The electron micrographs were also scanned for signs of prokaryotic endosymbionts that might be responsible for the DNR—none were found.

We have cultured both species of *Loxodes* in the laboratory. The cultures are not axenic but the cells of *Loxodes*, because of their large size and high specific gravity (barite inclusions¹¹), are easily separated from bacteria by gentle centrifugation and repeated re-suspension and washing in clean buffered media. Cells grown anaerobically were isolated in this way and assayed for nitrate reductase. The results from several assays are plotted in Fig. 2. Clearly nitrite production by each species is linearly

Table 1 Characteristics of the two *Loxodes* species investigated

	<i>Loxodes magnus</i>	<i>Loxodes striatus</i>
Cell volume (μm^3)	$1,136 \times 10^3 (51.4 \times 10^3; 40)$	$210 \times 10^3 (12.5 \times 10^3; 40)$
Cell protein (ng)	82.6(3.56; 22)	10.4(0.369; 23)
Nitrate reductase activity (ng $\text{NO}_2\text{-N}$ per cell per 40 min)	0.466(0.0195; 26)	0.0585(0.00223; 37)
<i>Loxodes magnus</i> mitochondria		
	Aerobic	Anaerobic
No. per unit volume (μm^3) of cytoplasm (N_v)	0.0405(0.00492; 35)	0.0684(0.00567; 53)
Surface area (μm^2) of inner mitochondrial membrane per unit volume (μm^3) of cytoplasm (S_v)	0.462(0.128; 49)	0.728(0.144; 45)
No. per cell	46.0×10^3	77.7×10^3

The two values given in parentheses are the s.e.m. and the number of measurements, respectively. Cell volumes were derived from measurements of the surface area of osmium-fixed cells compressed in a 20 μm deep Thoma chamber. Protein was determined using Sigma kit no. 690-A after solubilizing the material in 1% deoxycholate. The mean protein value for *L. striatus* is slightly lower than expected from measurements of cell size and is due to the highly vacuolar nature of the cytoplasm in this species, a feature which was included in cell volume measurements. N_v was determined by the stereological method described by Weibel¹⁴ using a size distribution constant (K) of 1.05, and shape constants (β) derived from measured mitochondrial axial ratios and Fig. 2.14 in Williams¹⁵. The number of micrographs examined exceeded the minimum sample size for point counting estimates derived using the progressive mean technique¹⁵. S_v was determined using the method described elsewhere¹⁵ with superimposed lines that completely cut the micrographs. The estimated numbers of mitochondria per cell were calculated from cell volume/ N_v .

Table 2 Bacterial contamination of washed *L. magnus*

Culture no.	No. of <i>Loxodes</i> in assay	No. of bacteria in assay MPN estimate	Total count ($\pm 95\%$ confidence limits)*	Nitrite produced in assay (ng NO ₂ -N per 40 min) (s.e.m.) (A)	Estimated bacterial nitrite production (ng NO ₂ -N per 40 min)† (B)	Estimated bacterial contribution to nitrite production (B/A) $\times 100$
1	765	ND(<10 ²)	1.06(± 0.11) $\times 10^4$	275 (2.82)	0-1.8	0-0.65%
2	1,131	ND(<10 ²)	0.36(± 0.04) $\times 10^4$	558 (14.7)	0-0.60	0-0.11%
3	299	ND(<10 ²)	0.43(± 0.05) $\times 10^4$	159 (4.21)	0-0.72	0-0.45%

Three washed suspensions of *L. magnus* from three different cultures were prepared as described in Fig. 2 legend. Part of each suspension was removed for enumeration of bacteria. The remainder was assayed in triplicate for DNR as described in Fig. 2 legend. The MPN (most probable number) estimate of nitrate reducers was determined using a dilution culture series incubated anaerobically¹⁶ with a dilution factor of 10 and eight replicates at each level. ND, no development of nitrate reducers after 14 days at 20 °C.

* The total count was obtained by counting all fluorescing bacteria observed on black membranes under incident UV light after staining with the fluorochrome, acridine orange¹⁷.

† Assuming that each bacterium present is alive, that 10¹⁰ bacteria constitute 1 mg protein¹⁸ and that all bacteria present are capable of reducing nitrate. Laboratory determined rates of bacterial nitrate reduction are highly variable but the value of 3 μ mol per min per mg protein we have used is at the upper end of the range¹⁹⁻²¹.

related to the number of cells in the assay and each relationship will pass through the origin. If we multiply the average DNR activities of the two cultured species of *Loxodes* by the cell densities recorded from the depth of maximum abundance on 14 July (Fig. 1; 1.38 m) the estimated total DNR activity obtained is 57.6 ng NO₂-N per 5 ml per 40 min. We have measured the retention of the *Loxodes* community on 30- μ m sieves to be 75%. This reduces the expected activity to 43.2 ng NO₂-N per 5 ml per 40 min, which is almost identical to the measured activity of 43.9 (Fig. 1). While such close agreement is probably fortuitous it does indicate that nitrite production by *Loxodes* alone can account completely for the *in situ* nitrate reductase activity measured in the >30 μ m fraction.

We also investigated the possibility that the nitrate reductase might be located within the cells of *Euglena* ingested by *Loxodes* as a food source. We could find no evidence for a nitrate reductase in axenic cultures of *Euglena* nor was there any

relationship between nitrite production per *Loxodes* cell and the quantity of ingested *Euglena*. Both of these findings are consistent with earlier reports¹². The mouthparts of *Loxodes* are adapted for feeding on relatively large particles and there is no evidence that *Loxodes* feeds on bacteria. We also determined the level of residual bacterial contamination in washed suspensions of *Loxodes* (Table 2). In three suspensions examined, from three separate cultures, the bacteria present would have made a negligible (maximum <1%) contribution to the measured DNR activity.

The endosymbiotic theory for the origin of mitochondria proposes that they arose from the invasion of bacteria into ancestral proto-eukaryotes¹³. The most likely candidate for the prokaryote ancestor is the present day nitrate-respiring bacterium, *Paracoccus denitrificans*⁸. The respiratory chains of *Paracoccus* and mitochondria are very similar except for certain adaptive features in the former, notably the presence of nitrate reductase. It is usually assumed that the facility for nitrate respiration has been lost from all present day mitochondria^{3,8}. It is of interest then that nitrate respiration can be attributed to the facultatively anaerobic protozoan *Loxodes*. This ciliate possesses many unusual features which make it atypical of ciliates in general—it is also very primitive. It is possible, of course, that its capacity for nitrate respiration has evolved recently. As an alternative hypothesis we propose that at least one of the adaptive features of the mitochondrial ancestor has been retained in this 'relict'⁷ protozoan, conferring on it the bacterial ability to switch between alternative electron acceptors and facilitating its cosmopolitan distribution⁷ in oxygen-free habitats.

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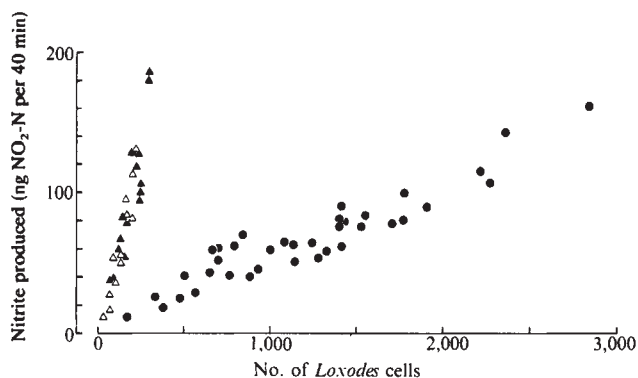


Fig. 2 Nitrate reductase activity in two species of cultured *Loxodes*: ▲ and △, *L. magnus*; ●, *L. striatus*. Data were obtained using cells taken from a large number of cultures. Cells were grown²⁷ microaerobically at 10 °C in the dark and fed axenically-grown *Euglena gracilis*; 20-24 h before assaying for nitrate reductase, cultures were stoppered with subseals, degassed with N₂/CO₂ (95:5) and charged with nitrate (final concentration >50 μ mol l⁻¹). Cells were isolated from the culture medium by gentle centrifugation (90g for 20 s) then washed twice by resuspension in phosphate buffer (pH 6.5). The final pellet of washed cells was homogenized and assayed for nitrate reductase as described in Fig. 1 legend. Alternatively, cells of *L. magnus* were isolated and washed with the aid of drawn-out Pasteur pipettes. Using a low power binocular microscope, cells were picked out individually and transferred through a series of four dishes containing clean medium. The final clean cells were then centrifuged and assayed in the normal way. As an additional control the final washings were also assayed for nitrate reductase; no activity was detected. The results obtained following the latter method of isolation (▲) are readily integrated with those obtained by the first method (△) described above.

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Chromosome abnormalities in human embryos after *in vitro* fertilization

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In vitro fertilization and embryo transfer is now an established treatment for certain forms of human infertility^{1–3}. High success rates have been reported following oocyte recovery and fertilization *in vitro*, the only major remaining difficulty being the high failure rate (80%) of implantation after replacement of the embryo into the mother. One possible reason for this failure of implantation is that some embryos may carry a lethal chromosome abnormality which does not allow development beyond the preimplantation stage. The need for information on the chromosomal status of early embryos fertilized and developed *in vitro* is recognized^{4,5} but two reported attempts to examine them have met with technical problems^{6,7}. Here we describe a method for examining chromosomes in 8-cell human embryos. Although we have made complete chromosome analyses for only three oocytes, our findings are surprising in that two of the three embryos were chromosomally abnormal. Data on the DNA content of the nuclei in a further eight cases showed that ~20% overall may be haploid.

Details of the volunteer egg donors are given in Table 1. One woman had experienced 6 yr of unexplained primary infertility (case 1) and six women were undergoing laparoscopic sterilization. The volunteer sperm donors were healthy males (their ages are listed in Table 1). All the participants were aware of the exact nature of the study and gave written permission for their gametes to be used. Oocytes were recovered from the ovaries of the women at laparoscopy by a method similar to that described by Renou *et al.*⁸, except that the gas phase in the pneumoperitoneum was nitrous oxide. In each patient, follicular development was induced with clomiphene (150 mg daily for 5 days) followed by 4,500 international units (IU) of human chorionic gonadotropin (HCG) intramuscularly 4 days after the end of clomiphene treatment. The follicles were aspirated 34.5–35.5 h after the HCG injection. Ultrasound was used 16 h before aspiration to locate the number, position and size of the follicles. Details of the follicles from which the oocytes were recovered are given in Table 1. After aspiration, the follicular fluid was transferred to a small portable incubator at 37 °C for the 3-min journey to the laboratory. There, the contents were immediately examined at room temperature and the cumulus mass containing the oocyte identified and transferred to a drop (0.9 ml) of culture medium under liquid paraffin in a small tissue culture dish (Falcon Plastics). The liquid paraffin had previously been equilibrated to the 5% CO₂ gas phase. Exposure to light was kept at the minimum necessary for accurate observation and manipulation. The dishes were incu-

bated at 37 °C in an atmosphere of 5% CO₂/5% O₂/90% N₂ for 7 h before insemination. Two types of culture medium were used: (1) Hams F10 (Flow) supplemented by 100 IU ml⁻¹ penicillin G, 0.5 mg ml⁻¹ streptomycin sulphate and 10% patient's serum which had been inactivated at 56 °C for 30 min (Table 1, cases 1a and b); and (2) Earles medium (Flow) supplemented by 0.0011 mg ml⁻¹ pyruvate, 100 IU ml⁻¹ penicillin G, and 10% inactivated patient's serum (cases 2–7). The semen samples were prepared in the standard way⁹ using the same medium as used for the egg, and the sperm were allowed to capacitate for 6 h at a concentration of 1 × 10⁷ ml⁻¹. Each oocyte was inseminated by adding 0.1 ml of the sperm preparation to the 0.9 ml drop of medium containing the oocyte to give a final sperm concentration of 1 × 10⁶ ml⁻¹. If more than one oocyte was recovered from a donor, they were inseminated at the same time using the same spermatozoa preparation.

Observations for cleavage stages were made twice daily but the number of pronuclei could not be determined because of the surrounding cumulus cells. The intervals between insemination and the first, second and third cleavages of the eggs were in agreement with the average timing of cleavage reported by others^{4,10}. The oocytes were transferred to fresh medium after the first division. About 70 h after insemination the embryos which seemed to have about eight blastomeres were transferred to culture medium containing colcemid (0.4 µg ml⁻¹) and left for 6 h. The embryos were then fixed (for method see Fig. 1 legend); the technique used produces metaphase plates of good quality for cytogenetic analysis, provided the zona pellucida is sufficiently dissolved to allow the metaphases to spread at fixation. The main problem is the small number of cells available. Clearly, more than one metaphase is preferable for accurate analysis, but the limited results below show that the early cleavages within the zygote are not necessarily synchronous.

Table 1 Details of follicles examined; ages of egg and sperm donors

Egg donor		Details of follicles			Sperm donor
No.	Age (yr)	Ovary	Volume (ml)	Concentration of oestradiol-17β (µM)	Age (yr)
(1)	35	a R	7.0	4.1	39
		b L	5.3	1.5	
(2)	31	L	5.5	3.5	31
(3)	36	L	7.0	2.7	27
(4)	39	a L	7.3	2.2	27
		b L	7.0	2.3	
		c L	5.5	1.5	
(5)	35	R	7.0	3.4	20
(6)	38	L	5.0	2.6	30
(7)	32	a R	3.0	3.2	39
		b R	2.0	1.2	

L, left and R, right ovary.

Table 2 Results of chromosome analyses

Oocyte	No. of interphase nuclei	Total no. of metaphases	No. analysable	Result
(1)a	3	4	3	22, X, -15
(1)b	4	2	1	46, XY
(2)	4	2	1	47, XY, +D
(3)	8	1	—	2N
(4)a	0	8	—	2N
(4)b	4	1	—	2N
(4)c	6	—	—	2N
(5)	9	—	—	N
(6)	8	—	—	2N
(7)a	12	—	—	2N
(7)b	11	—	—	2N

The results are summarized in Table 2. The chromosome complement in case 1a was 22, X, -15 (Fig. 1). Subsequent analysis of the karyotype of the egg donor and sperm donor from blood samples revealed that a normal chromosome variant—that is, large satellites on chromosome 13—found in the cleaving oocyte was identical to that found in the egg donor (see inset of Fig. 1). The haploid embryo nullisomic for chromosome 15 therefore contained only maternal chromosomes. The one cell available for analysis from case 1b was clearly 46, XY. In the oocyte from case 2, the blastomeres were less regular in size than in the previous two oocytes. Two mitoses were found, one of which was analysed as 47, XY, +D, but the preparation was of insufficient quality to allow the extra D to be identified. The other metaphase was broken but the scattered chromosomes lay in a completely different section of the slide. Oocytes 3 and 4b each had one mitosis which could not be analysed accurately, but which was clearly in the diploid range. In case 4a there were eight metaphases, but they were not well spread and were difficult to analyse. Two of the cells contained 45 chromosomes, one with a chromosome 15 missing, another contained 44 and one possibly 46 chromosomes, however the observations were too ambiguous to allow an accurate result. In the remaining five oocytes, all the nuclei were in interphase; these preparations were examined for their DNA content by Dr Adrian Sumner (MRC Clinical and Population Cytogenetics Unit, Edinburgh). He found, by using DNA microfluorimetry and staining with ethidium bromide, that in one preparation—case 5—the DNA content of the nuclei was in the haploid range, and that of the remaining four was in the diploid range.

A surprising and remarkable finding is that of the 11 embryos examined for their ploidy, 2 appeared to be haploid, and of the 3 examined chromosomally, 2 showed evidence of nondisjunction of an autosome. In one, in addition to its haploid state, there was nullisomy 15, and in the other, trisomy D. Haploidy in early embryonic development in man is almost unknown, although it occurs widely in other mammalian species either spontaneously or through artificial induction (for review see ref. 11). This suggests that mammalian oocytes have an inherent tendency to divide and that this tendency is merely promoted by fertilization or a parthenogenetic stimulus. In our case, the oocytes were not, as far as we are aware, exposed to any of the factors known to cause spontaneous activation of mammalian eggs. In fact, oocytes 1a and 1b from the same woman were treated identically, were inseminated with the same sperm preparation, then cleaved at the same rate and were completely indistinguishable from each other in appearance at every stage. Clearly, the 46, XY embryo developed from an oocyte with a normal chromosome complement of 23, X, whereas the haploid embryo must have arisen from an oocyte nullisomic for chromosome 15. Monosomy for an autosome has been recorded for a G group chromosome where it occurs with a frequency of 0.01% in spontaneous abortions. Monosomies of the other autosomes have not been found although their occurrence as a natural corollary to trisomy has been discussed frequently and they have been assumed to be incompatible with survival even to the stage of recognizable pregnancy¹². Our results show that eggs nullisomic for chromosome 15 are viable to the eight-cell stage at least.

Trisomy for a D group chromosome is also an uncommon chromosome abnormality in clinically recognized pregnancies. Trisomy 13, but not trisomy 14 or 15, has been recorded in surveys of live newborn and its incidence is 0.01% (ref. 13). Trisomies 13, 14 and 15 have all been reported in surveys of spontaneous abortions where they have been found in 6% of the abortions that are chromosomally abnormal¹⁴. They are all associated with a significantly increased maternal age¹⁵. Note that the mean maternal age (35.1 yr) of our egg donors is high and therefore the group carries a greater risk of nondisjunctional errors occurring than in women of mean reproductive age.

There are now extensive data on the incidence (~50%) of chromosome anomalies in spontaneous abortions from clinically recognized pregnancies in man, but no information on

their incidence in preimplantation embryos. Recent work^{16,17} has shown considerable loss of early pregnancies as detected by analysis of urinary HCG in women trying to conceive. Edmonds *et al.*¹⁷ concluded that ovulatory cycles exposed to the risk of pregnancy carry a 59.6% chance of conception and 56.8% risk of embryonic loss. This fecundity of ~25% agrees remarkably well with clinical and demographic data reviewed by Short¹⁸. The contribution of lethal chromosome anomalies to very early pregnancy loss *in vivo* is unknown. Two previous attempts to karyotype *in vitro* fertilized human embryos have

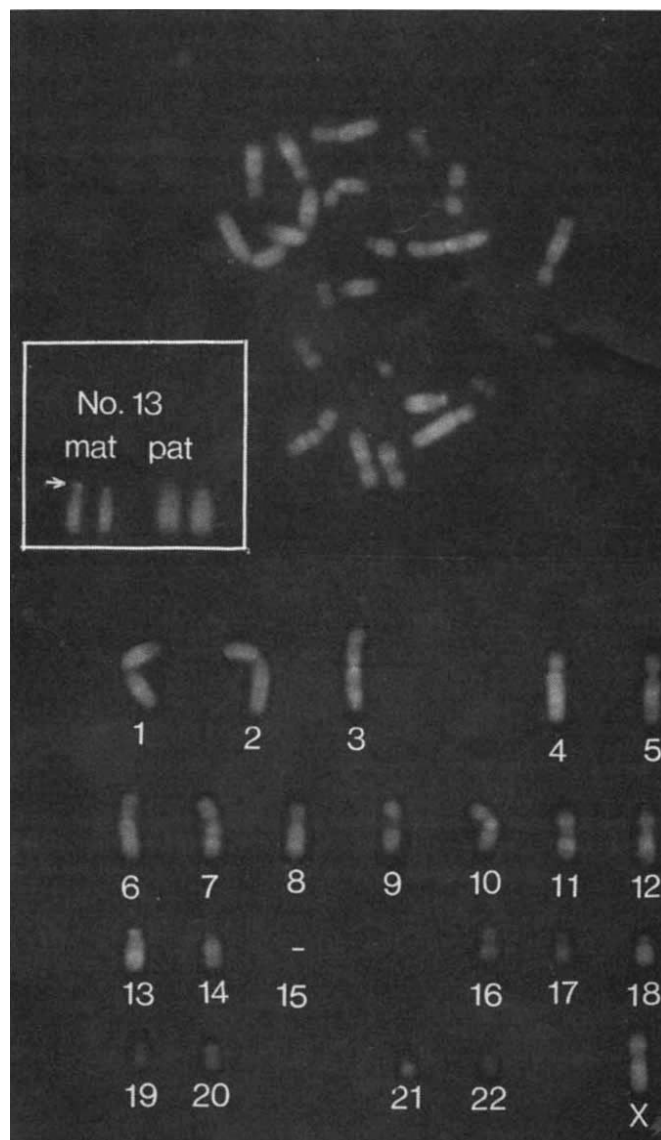


Fig. 1 Karyotype of an eight-cell human embryo having a 22, X, -15 chromosome complement. Inset shows large satellites on one no. 13 chromosome in egg donor. The technique of fixation was basically that described by Tarkowski¹⁹ but modified because the zona pellucida in humans is tougher than that in the mouse. The embryo was transferred to a solution of pronase (0.2 mg ml⁻¹) in phosphate-buffered saline for 7 min at room temperature to partially dissolve the zona pellucida, then transferred to a hypotonic solution of KCl (0.075 M) for 8 min before being placed on a clean slide together with a small drop of KCl solution. A drop of fresh fixative (glacial acetic acid/methanol, 1:3) was dropped onto the egg from a height of ~1 cm. At this stage the blastomeres could be observed to separate and spread over a small area in the vicinity of the spreading drop. A second drop of fixative was added as before, then the slide was fanned vigorously to aid flattening and drying of the cells. The slides were stained with the fluorescent stain spermidine bis-acridine (0.005%) and examined with a Leitz Ortholux microscope using a 50-W mercury lamp, a BG 12 exciter and a 510-nm barrier filter.

been reported: Edwards⁶, who examined chromosomes primarily to exclude triploidy, found 15 embryos to be "approximately diploid". Wramsby⁷ looked at three oocytes, two before cleavage and one which had cleaved at 40 h and found that the latter had a haploid set of 23 or 24 chromosomes. Further chromosome data are clearly needed. Apart from their clinical relevance, such data might also elucidate why natural fecundity in man is so low.

It must be emphasized that over 100 babies have now been born after *in vitro* fertilization without any apparent chromosome abnormality. Chromosome abnormalities of the kind we have found clearly result in early embryonic loss, and probably contribute to the high failure rate after embryo transfer.

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Sex reversal in XY mice caused by dominant mutation on chromosome 17

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A Y-linked gene(s) is undoubtedly a prerequisite for testicular development in mammals. However, exceptional cases suggest that the mere presence of the Y chromosome or the Y-linked testis-determining gene does not totally control the fate of the bipotential gonad¹. For example, in the mouse, a major as yet unmapped autosomal locus is necessary for normal testicular development and at least one additional autosomal locus is implicated². We present here a third autosomal sex-determining locus. This dominantly inherited trait, tentatively named T-associated sex reversal (gene symbol *Tas*), is closely linked to or a part of the *T/t* complex on chromosome 17 of the mouse. Gonads of chromosomally XY individuals who inherit *Tas* on the C57BL/6J inbred strain background differentiate as ovaries or ovotestes.

The *Tas* mutation was noted in our hairpin-tail (T^{hp}) stock, which was produced by repeatedly introducing the T^{hp} gene into the C57BL/6J inbred strain. T^{hp} occurred spontaneously in the AKR/J inbred strain³. Like other dominant mutations at the *T* (brachyury) locus, T^{hp} shortens tails of heterozygotes,

Table 1 Breeding data of eight externally normal $T^{hp}/+$ males

Sire no.	Generation on C57BL/6J	Classification of gonads at autopsy		Classification of weanlings for external phenotypic sex and T^{hp}			
		Right	Left	Females +	T^{hp} +	Males +	T^{hp} +
29	N4	T<N	T-N	18	10	15	4
32	N5	T-N	OT	20	13	11	3
35	N5	T-N	OT	17	4	17	5
42	N5	T-N	OT	18	8	8	2
37	N6	T-N	T<N	17	1	15	5
38	N6	T-N	T<N	16	8	2	1
45	N6	T-N*	?	0	0	0	0
48	N6	?	T-N*	0	0	0	0
Total offspring				106	44	68	20

T-N = normal testis; T<N = testis smaller than normal; OT = ovotestis; T-N* = testis normal, no seminal vesicle; ? = ovary-like structure in fat pad located near kidney (normal ovarian position).

causes embryonic death in the homozygote and interacts with *t* alleles in the T^{hp}/t individual to produce a tailless phenotype. In addition, T^{hp} behaves as a genetic deletion: hairpin-tailed offspring produced from the mating of a $T^{hp}/+$ male and *qk/qk* female (*qk* is quaking, a recessive gene on chromosome 17) have a quaking phenotype⁴. A unique feature of hairpin-tail is that, with rare exceptions (unpublished data), embryos receiving T^{hp} from the maternal genome die *in utero* or at birth^{5,6}.

Although abnormalities of sexual development have not been previously ascribed to the $T^{hp}/+$ phenotype, at the fourth and fifth generations of backcrossing T^{hp} onto the C57BL/6J inbred strain genome, we noted two $T^{hp}/+$ mice with mixed external sex characteristics including underdeveloped penis and scrotum and mammae-associated pigment, the latter feature being normally restricted to females. These animals were karyotyped as XY and found at autopsy to be true hermaphrodites, each having a testis (or ovotestis) and a contralateral ovary.

We analysed the breeding records of six fertile $T^{hp}/+$ males in our C57BL/6J- T^{hp} colony for segregation of T^{hp} , sex ratio and further evidence of hermaphroditism (Table 1). Irrespective of sex, the number of normal-tailed offspring (174) exceeded the number of hairpin-tailed offspring (64), suggesting a loss of $T^{hp}/+$ individuals. The number of phenotypically female normal-tailed (106) and hairpin-tailed (44) offspring exceeded the number of male offspring (68 and 20, respectively), indicating that the sex ratio within the T^{hp} stock was skewed towards female offspring. Although none of the six fertile males showed external sexual abnormalities, macroscopic inspection at autopsy of these males and two sterile $T^{hp}/+$ males revealed that gonadal development was not normal: in three fertile males the left gonad was an ovotestis; in three fertile males one testis was normal in size but the contralateral testis was smaller, suggestive of an ovotestis; and in the two sterile males one gonad, although necrosed, was presumed to have been an ovary because it was located in a fat pad at the posterolateral pole of the kidney.

A protocol based on previous studies was implemented to determine if the skewed sex ratios observed at weaning and the anomalous sexual development had a genetic basis^{7,8}. Ninety-five fetuses from matings of normal C57BL/6J females to C57BL/6J $T^{hp}/+$ males were classified at 14-15 days of development for gonadal sex, sex chromosome complement and T^{hp} phenotype. As seen in Table 2, we obtained the expected 1:1 ratio of female to male normal-tailed fetuses. This observation and a normal sex ratio of normal-tailed offspring (100♀:114♂) from current matings involving C57BL/6J $T^{hp}/+$ males to C57BL/6J females obviates the significance of the excess of normal-tailed females recorded in Table 1. Of the 25 normal-tailed XY fetuses, 18 had two normal testes and 7 had at least one testis with a small area of delayed testicular development at one or both ends (see Table 2).

Table 2 Classification of 95 fetuses, 14–15 days of age*, from matings of C57BL/6J females to C57BL/6J $T^{hp}/+$ males

T^{hp} genotype	Ovaries			Testes		Ovary and ovotestis	Ovotestes
	+/+	$T^{hp}/+$	$T^{hp}/+$	+/+	$T^{hp}/+$	$T^{hp}/+$	$T^{hp}/+$
Sex chromosomes	XX	XX	XY	XY	XY	XY	XY
No. of fetuses	23	20	15†	25‡	0	7	5

* Analysis at this stage of development¹⁴ compensates for undetected neonatal losses and ensures accurate identification of hermaphroditic individuals.

† Although adults from this class appeared to be normal phenotypic females, to date, no offspring have been obtained when these XY females are mated to normal C57BL/6J males. Adult $T^{hp}/+$, XY females are positive for the H-Y transplantation antigen¹⁵.

‡ One or both testes of seven fetuses had 'ovarian-like' areas at the caudal or cranial ends. Histological analyses of these areas did not show the characteristic features of ovarian tissue, as was the case for the ovotestes from the 12 $T^{hp}/+$ hermaphrodites.

Hairpin-tailed and normal-tailed fetuses were found in the expected 1:1 ratio, confirming later death of $T^{hp}/+$ mice, as suggested from our weaning data and perinatal data reported previously⁵. Further analysis of the 47 hairpin-tailed fetuses revealed that 12 were chromosomally XY and true hermaphrodites, each having an ovary and ovotestis, or two ovotestes (Fig. 1). Ovarian tissue comprised 50% or more of each ovotestis and was located at the cranial and caudal ends, as is characteristic of tissue organization in ovotestes in two other inherited murine hermaphroditisms^{7,8}. The remaining 35 $T^{hp}/+$ fetuses had ovaries; however, chromosome analyses revealed that 15 of these fetuses were karyotypically XY. If we add the group of XY $T^{hp}/+$ female fetuses to the XY $T^{hp}/+$ hermaphroditic fetuses, we obtain the expected 1:1 ratio of XX to XY $T^{hp}/+$ individuals. The most striking conclusion from the fetal analysis was that no $T^{hp}/+$ XY individual had testes; all were partially or completely sex-reversed.

Our data show that the sex-reversal phenomenon is inherited as a dominant autosomal trait on chromosome 17. At least three relationships to T^{hp} are possible. First, the observed sex reversal may be a pleiotropic effect of the T^{hp} gene, with maximum (that is, easily detectable) penetrance on a C57BL/6J background. Second, because the deleted and therefore functionally null chromosome 17 segment involved in T^{hp} allows the expression of any genes within the corresponding area on the normal chromosome 17^{4,9}, sex reversal may be the result

of hemizygous gene expression of a locus from the C57BL/6J genome. This hypothesis implies that the homozygous state of this locus results in normal male sexual differentiation, as sex reversal is not found in the C57BL/6J inbred strain *per se*. Preliminary analyses provide data to support either of the above interpretations. After three generations of transferring the T^{hp} mutation from the C57BL/6J- T^{hp} congenic strain to the C3H/HeJ inbred strain we observe no unusual sex ratios (26 ♀ +/+; 7 ♀ $T^{hp}/+$; 17 ♂ +/+; 16 ♂ $T^{hp}/+$). Furthermore, no XY females or hermaphrodites were found among 17 (14–15-day old) $T^{hp}/+$ fetuses (6 XX ♀; 11 XY ♂) obtained from the initial outcross of a C57BL/6J $T^{hp}/+$ male to C3H/HeJ females. This suggests that, unlike the C57BL/6J inbred strain, the C3H/HeJ genome either does not permit the expression of sex reversal or the hemizygous expression of the putative sex-determining gene on chromosome 17 from C3H/HeJ results in normal gonad development.

A third possibility is that the T^{hp} -associated sex reversal trait is a closely linked *de novo* mutation. Although we did not find the reciprocal recombinant classes (hairpin-tailed XY males with testes, normal-tailed XY females with ovaries and normal-tailed XY hermaphrodites) indicative of a separate mutation, the sampling of a much larger fetal population may yield such recombinants and thus implicate a closely linked mutation.

Although we are unable to show that the sex reversal associated with T^{hp} is a separate locus, we are designating this trait T-associated sex reversal (*Tas*) to acknowledge its singular importance to the understanding of primary sex determination. As a testis-determining gene, the normal allele of the *Tas* gene may be required for normal testicular differentiation to proceed beyond the step initiated by the Y-linked testis-determining gene (*Tdy*)², relegating *Tdy* to a primary but not the exclusive testis-determining locus¹. On the other hand, *Tas* may be a mutation affecting a critical step in ovarian differentiation, a process traditionally considered to be automatic (without primary genetic control) in the absence of *Tdy*^{10,11}. We propose that the *Tas* locus could function as the ovarian determination gene. The normal allele would initiate ovarian development in the XX individual but would be inactive in the XY individual when *Tdy* functions to initiate testicular differentiation. If the *Tas* locus is not functioning properly and is active in the XY individual before or concurrent with *Tdy*, the gonads of XY individuals would differentiate as ovaries or ovotestes, respectively, as in our C57BL/6J- T^{hp} stock.

Whether *Tas* proves to control a genetic step in ovarian or testicular differentiation, the discovery of a primary sex-determining gene on mouse chromosome 17 provides an intriguing addition to the loci in the *T/t* complex already known to influence development and fertility^{4,12,13}.

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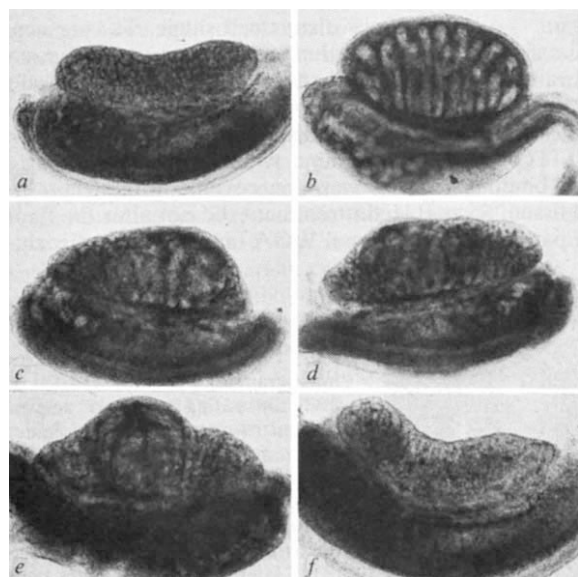


Fig. 1 Freshly dissected gonads from 14–15 day fetuses. *a*, Ovary from XX C57BL/6J fetus. *b*, Testis from XY C57BL/6J fetus. *c*, *d*, Ovotestes from an XY $T^{hp}/+$ individual. Note the reticular appearance of ovarian tissue at the cranial and caudal and the thickened capsule over central testicular areas¹⁶. *e*, *f*, An ovotestis and ovary, respectively, from a second XY $T^{hp}/+$ fetus.

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Basement membrane polarizes lectin binding sites of *Drosophila* larval fat body cells

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Vertebrate epithelial cells in monolayers are asymmetrical in that their apical and basal membranes differ in morphology and function^{1–3}. That this cell polarity depends on the presence of tight junctions can be demonstrated by labelling one surface of a cell monolayer in culture with fluorescent lectins and lipid probes, and subsequently observing whether the labels disperse to the opposite cell surfaces^{4,5}. Here we report on a differential distribution of binding sites for the lectin wheat germ agglutinin (WGA) on the cells of *Drosophila melanogaster* larval fat body, and show that the pattern is correlated with the structural association between the cell surfaces and their overlying basement membrane.

The larval fat body of *D. melanogaster* is bilaterally symmetrical, and is organized in a continuous sheet one cell thick. This sheet of adipose cells is covered by extracellular matrix, basement membrane, and is suspended in the haemocoel. Cell shape and its topography with respect to the basement membrane depend on the position of the fat body cell within the sheet^{6,7}. Cells located centrally are columnar, with polygonal surfaces apposing the basement membrane, whereas cells from the tissue margins are wedge-shaped with at least three sides covered by basement membrane (Fig. 1a–c). In addition to

these, the cells forming the commissures that are chains of single cells have a roughly cylindrical shape and only their two ends that are in contact with neighbouring cells in the string are not covered by basement membrane while a cell located at a distal tip of the tissue is toe-shaped, with all its surfaces covered by basement membrane except that surface linking it to its neighbour in the tissue. Thus, each fat body cell has some surface that makes contact with other fat body cells while its remaining surfaces facing the haemocoel are covered by basement membrane.

When freshly dissected larval fat bodies in Ringer solution are treated with fluorescein isothiocyanate-conjugated WGA (FITC-WGA), the tissue surfaces light up with fluorescent specks (Fig. 2a). If the FITC-WGA is pretreated with chitobiose, its haptenic sugar, it fails to bind to the fat body surfaces. To determine whether FITC-WGA was bound to the basement membrane, to the cell surfaces beneath it, or to both, we treated fat bodies on microscope slides with FITC-WGA, rinsed them with distilled water, placed a coverglass over the tissues, and applied gentle pressure, squeezing the cell contents from the lipid-laden cells to free the basement membrane sheets. The coverglass was then lifted slightly and the membranes thoroughly flushed with several changes of distilled water to remove cellular debris. Such preparations showed intense fluorescence in the areas overlying cell surfaces and only slight fluorescence in the regions corresponding to intercellular spaces (Fig. 2b). This differential distribution of WGA binding suggests either that basement membrane overlying cell surfaces contains more lectin binding sites than intercellular regions or that the cell membranes which also contain WGA binding sites remain adherent to the basement membrane during preparation of the samples. The latter possibility is the more likely because osmotic disruption of the cells obviously releases their internal contents but may not be effective in dislodging the cell membranes from the basement membrane.

To examine the distribution of lectin binding sites on fat body cell surfaces free of basement membrane, we first reacted the fat bodies with FITC-WGA and then dissociated the cells using collagenase⁸. Each dissociated cell retained the shape that it originally had in the intact tissue. We presume this result to be due to the high concentration of lectin used, as we previously found⁹ that 50–200 $\mu\text{g ml}^{-1}$ of WGA immobilizes (fixes) surface structures of *Drosophila* cells in culture whereas low (5–10 $\mu\text{g ml}^{-1}$) concentrations distort cell shape. The surfaces of the dissociated fat body cells that had been covered by basement membrane remained intensely fluorescent while the lateral surfaces that had been in contact with neighbouring cells lacked fluorescence (Fig. 2c). These dissociated cells were re-treated with FITC-WGA to determine whether the lateral surfaces contain binding sites that were inaccessible to the lectin in the intact tissue. Second lectin treatment did not alter the fluorescence patterns; no additional WGA binding was noticeable at

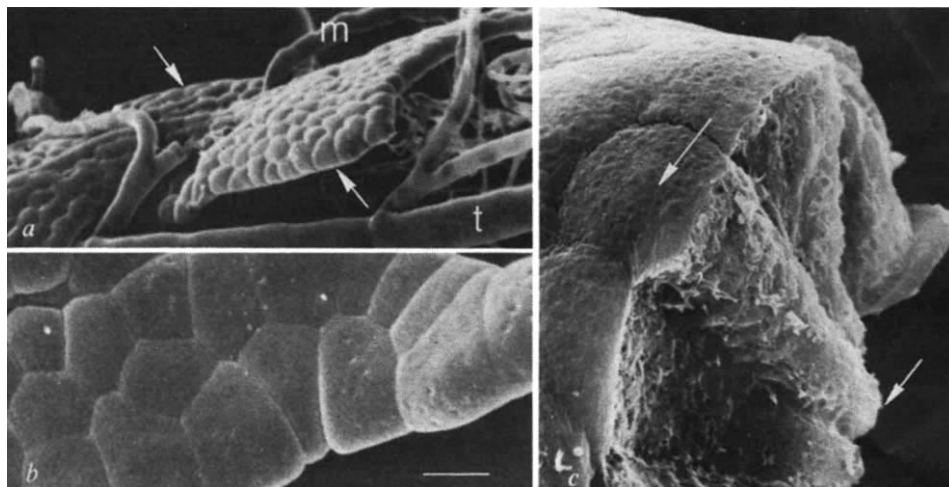
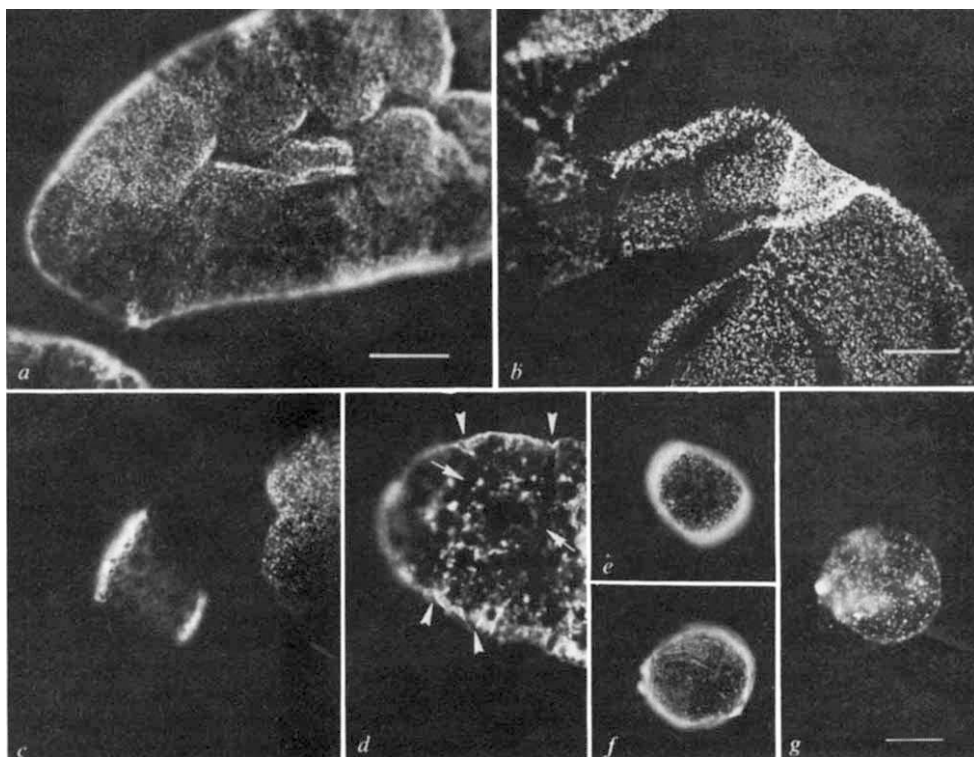


Fig. 1 a, A scanning electron micrograph of a portion of larval fat body illustrating a sheet of adipose cells (arrows); trachea, t; malpighian tube, m. b, A slightly higher magnification of another region of fat body showing the shape of cells in the centre and at the margins of the tissue. Scale bar, 50 μm . c, View of an intercellular face of fat body fractured by a micromanipulator interfaced with the scanning electron microscope⁷. The arrows indicate the two cell surfaces covered by basement membrane.

Fig. 2 *a*, FITC-WGA binding at the surfaces of an intact region of fat body, comparable to that in Fig. 1*b*. Freshly dissected tissue was rinsed with Ringer solution and treated with $100 \mu\text{g ml}^{-1}$ FITC-WGA in Ringer at room temperature for 3 min. The outlines of the individual cells are apparent. Scale bar, $50 \mu\text{m}$. Same magnification for *b-d*. *b*, FITC-WGA binding to a basement membrane preparation of a commissure region obtained by squashing and rinsing freshly dissected tissue to remove intracellular contents. WGA binding is concentrated over cellular regions and sparse at intercellular regions. *c*, Lateral view of a cell isolated from fat body that had been treated with FITC-WGA before incubation with collagenase. WGA binding is limited to the cell surfaces formerly covered by basement membrane and facing the haemocoel. The orientation of this cell corresponds to that of the indicated cell in Fig. 1*c*. The polar view of three cells, to the right, compares with the surface view of cells in tissue in *a*. *d*, Frozen section of a fat body cell (arrowheads) showing FITC-WGA binding at its two external surfaces and not its internal surfaces (arrows). This cell, unlike the bulk of the cells in the frozen sections, is sufficiently separated from its neighbours for the intercellular margins to be clearly visible. Intracellular binding also occurs in sectioned material. *e*, A fat body cell which was first isolated by treating the tissue with collagenase and then reacted with FITC-WGA. The fluorescent halo is an optical effect which is not so pronounced in *f* which shows the same cell after it has been rolled over and slightly flattened. FITC-WGA is dispersed over the cell surfaces and not limited to some surfaces as in *c* and *d*. *g*, A fat body cell from a pupa shows FITC-WGA binding sites as small specks over its surface. Large intracellular protein globules are autofluorescent. Scale bar, $50 \mu\text{m}$; same magnification for *e* and *f*.



the intercellular surfaces exposed by dissociating previously labelled fat body.

The preceding observations suggest that most of the lectin binding sites of the cell surfaces are concentrated at those surfaces facing basement membrane. It is, however, possible that exposure to collagenase and the subsequent washing required to remove the enzyme modifies WGA binding sites. Therefore, frozen sections of fat body were first lightly fixed in 2% buffered paraformaldehyde and then treated with FITC-WGA to assure that lateral surfaces and exterior surfaces were simultaneously exposed to the lectin. Figure 2*d* confirms that WGA binding sites are more heavily concentrated at the cell surfaces in apposition to basement membrane.

To visualize the distribution of WGA binding sites over the cell surfaces and basement membrane as well as to establish that WGA penetrates the basement membrane and binds to the underlying cell surfaces, we treated fat bodies with peroxidase-conjugated WGA (P-WGA) or ferritin-conjugated WGA (F-WGA), and examined epon sections in the transmission electron microscope. As a control for the specificity of WGA binding, fat bodies were incubated with P-WGA or F-WGA that had been pretreated with chitobiose. The peroxidase reaction was intense at the cell surfaces and basement membrane but negative in the controls (Fig. 3*a, b*). Likewise, ferritin particles were distributed in the basement membrane and the underlying cell surfaces, but none were found in the controls, excluding the possibility of nonspecific entrapment of ferritin in fat body basement membrane. An additional control was omission of osmium post-fixation and staining of sections with vanadatomolybdate¹⁰ rather than uranyl acetate-lead citrate. No apparent differences in basement membrane ultrastructure were detected among the groups, but fixation of intracellular components was poor when osmic acid was omitted.

The basement membrane surrounding the fat body of a mid-third instar larva is 40–60 nm thick. At low magnification

it appears amorphous, but at higher magnifications ($\times 50,000$ – $100,000$) a fibrillar network can be seen. Its mesh-like nature is best viewed in tangential sections which also reveal that fine fibrillar attachments extend from the fat body cell surfaces into the basement membrane net (Fig. 3*c*). Figure 3*c, d* also illustrates the disposition of F-WGA in the basement membrane network and on the surfaces of the underlying fat body cell membranes. The surfaces of fat body cells beneath basement membrane are highly convoluted¹¹, and ferritin particles are distributed along all these surfaces.

During the pupal period, basement membrane of the larval fat body is dissolved and the dissociated individual adipose cells round up and disperse within the pupal haemocoel¹². When these dissociated cells are washed with Ringer solution and treated with FITC-WGA, the binding sites appear as minute fluorescent specks scattered over the surfaces (Fig. 2*g*). None of the cells from the pupa show an asymmetrical distribution of binding sites, suggesting that reorganization of the fat body cell surface occurs when the cells are freed from the tissue masses. This reorganization, which involves cell shape, must also include redistribution of the lectin binding sites at the cell surface.

Dissolution of fat body basement membrane and rounding of the free cells can be mimicked *in vitro* by incubating freshly dissected larval fat bodies with collagenase and thoroughly rinsing the freed cells with Ringer solution⁸. When experimentally rounded fat body cells are treated with FITC-WGA, their surfaces light up in a pattern resembling that of pupal cells (Fig. 2*e, f*). Fluorescent specks are distributed over the cell surfaces rather than asymmetrically concentrated in some areas.

In conclusion, our studies demonstrate that *Drosophila* larval fat body cell membranes are polarized, as indicated by a heavy concentration of WGA binding sites on those surfaces covered by basement membrane and facing the haemocoel. We suggest

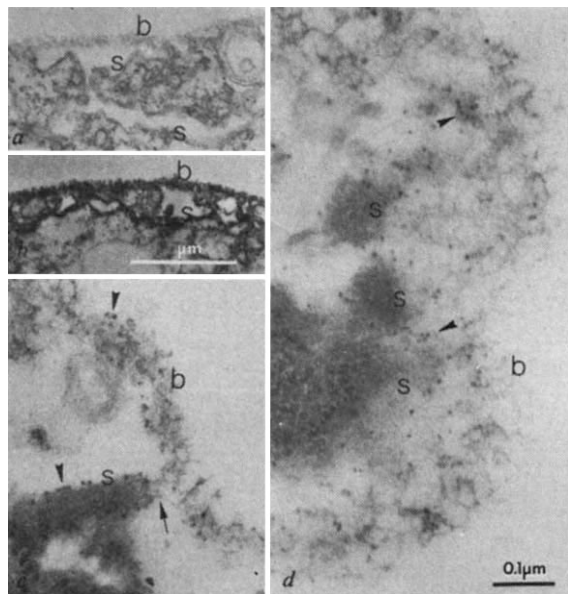


Fig. 3 *a*, A transmission electron micrograph (TEM) of fat body treated with P-WGA that had been preincubated with chitobiose. This is the control for *b*; basement membrane, *b*; fat body cell surface, *s*. Scale given in *b*. *b*, TEM of fat body treated with P-WGA showing the intense peroxidase reaction in the basement membrane and the cell surface. Note also that the fat body cell surfaces are highly convoluted, a factor that may account for the speckled appearance of the cells treated with FITC-WGA, because at some points, such as the surfaces marked *s* in *a*, there will be a triple layer of FITC-WGA excited by UV light and viewed from above. *c*, TEM of a small region of fat body surface treated with F-WGA. This selected area illustrates fine fibrous connections (arrow) between the fat body cell surface, *s*, and the overlying basement membrane, *b*. The arrowheads indicate some of the ferritin particles in the basement membrane and on the cell surface. Magnification same as *d*. *d*, A tangential section of basement membrane treated with F-WGA showing the fibrillar network constituting an open structure. The arrowheads point to some of the ferritin particles. The electron-dense material to the left is through the surface, *s*, of the fat body cell. Tissue fixed in buffered formaldehyde-glutaraldehyde; section stained in vanadatotomybdate.

that a structural association of the cell membrane and the overlying basement membrane stabilizes this orientation of cell-surface molecules. When the basement membrane is lost during the pupal stage, the cytoskeleton must rearrange as the cells assume a spherical shape. Concomitant with this reorganization is the loss of cell-surface polarity as it exists in the intact tissue and a redistribution of lectin binding sites of the cell surface. Note also that pretreatment of fat body tissue with WGA must immobilize cytoskeletal elements underlying the cell surfaces so that the fat body cells dissociated subsequently retain their original shapes.

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T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy

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In some subjects the infective agent of leprosy, *Mycobacterium leprae*, causes disseminated (lepromatous) disease. Such subjects have a major role in the transmission of the disease and show deficient T-cell responses both *in vivo* and *in vitro* to *M. leprae*, but not to other antigens¹⁻⁴. Numerous studies have recently shown that T cells with functional capabilities after initial triggering with antigen can be maintained in a state of continuous proliferation *in vitro* when cultured in medium containing interleukin 2 (IL-2)⁵⁻⁸. Here we have studied the effect of IL-2 rich T-cell conditioned medium on lepromatous peripheral blood mononuclear cells. Our results show that although lepromatous T cells fail to produce IL-2 after exposure to *M. leprae* they can respond by proliferation to *M. leprae* in the presence of T-cell conditioned medium, suggesting that the unresponsiveness in lepromatous leprosy results from a deficiency in the production of IL-2 or related factors and not a lack of *M. leprae*-reactive T cells.

As shown in Table 1, peripheral blood mononuclear cells (PBMC) of lepromatous patients [4 with the borderline form (BL) of the disease, 13 with the lepromatous (LL) form], responded poorly, as expected, to *M. leprae* in culture. The mean stimulation index (SI) was 1.13 ± 0.15 . However, the specificity of this unresponsiveness is shown by their response to purified protein derivative (PPD) (SI mean 5.41 ± 1.47). The background ³H-thymidine incorporation varied and was very high in some patients, for example patients 8 and 9. The reason for this remains unclear, but may be related to bacterial load, as these two patients had the highest bacteriological index (BI) in the group. T-cell conditioned medium (TCM) alone failed to stimulate DNA synthesis in most of the patients. On the otherhand, when TCM was added with *M. leprae*, the SI increased in all the patients (mean 8.95 ± 2.28). In patients 3, 8, 9 and 11, this increase was due to a decrease in background and is therefore of questionable significance, but in the others it was due to an increased response to TCM+*M. leprae*. Moreover, in 13 patients (3 BL, 10 LL) TCM also clearly increased the response to *M. leprae* as compared with *M. leprae* alone.

These observations do not appear to be due to phytohaemagglutinin (PHA) present in TCM, because control supernatants prepared from PBMC in the absence of PHA, but reconstituted with PHA in concentrations analogous to that of TCM, were found to be without effect.

As shown in Table 2, lepromatous patients also failed to produce IL-2 in response to *M. leprae*. The effect of TCM and *M. leprae* on PBMC from subjects not exposed and not responsive to *M. leprae* alone was also studied. As shown in Table 3, such subjects (Norwegian blood donors) remained unresponsive to *M. leprae* in the presence of TCM. Thus, the effect observed

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Table 1 Effect of T-cell conditioned medium on DNA synthesis in peripheral blood mononuclear cell cultures (PBMC) of lepromatous patients to *M. leprae*

Patient no.	T-cell conditioned medium <i>M. leprae</i>			³ H-thymidine incorporation (c.p.m. $\pm$ s.d.)						PPD	
	Histo-pathological diagnosis	Bacteriological index	Duration of treatment (yr)*	+	+	-	+	-	-	SI	SI
				1	2	SI	3	4	SI		
1	BL	5	1/12	15.573 $\pm$ 5.973	3.391 $\pm$ 1.363	4.6	3.568 $\pm$ 1.071	4.006 $\pm$ 2.071	0.9	13.536 $\pm$ 5.712	3.4
2	BL	4.8	3/12	41.427 $\pm$ 5.269	7.946 $\pm$ 2.461	5.2	4.262 $\pm$ 1.789	6.481 $\pm$ 781	0.7	13.541 $\pm$ 645	2.1
3	BL	3	3/12	15.644 $\pm$ 1.069	4.521 $\pm$ 117	3.5	10.371 $\pm$ 210	8.427 $\pm$ 1.769	1.2	30.278 $\pm$ 1.857	3.6
4	BL	3.2	5	38.137 $\pm$ 4.312	3.374 $\pm$ 564	11.3	3.008 $\pm$ 671	3.980 $\pm$ 1.001	0.8	20.009 $\pm$ 5.628	5.0
5	LL	4	0	88.886 $\pm$ 4.156	13.937 $\pm$ 2.854	6.4	1.743 $\pm$ 960	3.260 $\pm$ 1.962	0.5	20.035 $\pm$ 4.133	6.2
6	LL	5	0	91.719 $\pm$ 5.293	2.547 $\pm$ 1.477	36.0	3.153 $\pm$ 1.647	2.152 $\pm$ 716	1.5	4.492 $\pm$ 868	2.1
7	LL	5	0	10.937 $\pm$ 547	4.881 $\pm$ 1.899	2.2	3.149 $\pm$ 11	3.395 $\pm$ 1.371	0.9	21.574 $\pm$ 99	6.4
8	LL	6	1/12	22.806 $\pm$ 1.899	7.919 $\pm$ 24	2.9	23.817 $\pm$ 1.919	17.334 $\pm$ 2.580	1.4	27.036 $\pm$ 3.416	1.6
9	LL	6	2/12	13.083 $\pm$ 3.616	5.574 $\pm$ 1.418	2.4	42.026 $\pm$ 6.980	21.012 $\pm$ 3.001	2.0	15.700 $\pm$ 217	0.8
10	LL	5.2	2/12	90.769 $\pm$ 2.078	12.628 $\pm$ 6.626	7.2	3.128 $\pm$ 390	8.421 $\pm$ 768	0.4	17.769 $\pm$ 2.068	2.1
11	LL	5	6/12	4.453 $\pm$ 899	1.278 $\pm$ 1.188	3.5	4.857 $\pm$ 1.400	2.704 $\pm$ 1.245	1.8	25.670 $\pm$ 6.900	9.5
12	LL	2	2	20.708 $\pm$ 6.363	1.536 $\pm$ 270	13.5	1.288 $\pm$ 726	3.752 $\pm$ 2.206	0.3	19.313 $\pm$ 1.077	5.2
13	LL	3	2	99.556 $\pm$ 5.651	3.617 $\pm$ 1.167	27.5	1.837 $\pm$ 582	778 $\pm$ 550	2.4	20.799 $\pm$ 5.711	26.7
14	LL	4	2	34.932 $\pm$ 8.099	2.735 $\pm$ 1.463	12.8	2.681 $\pm$ 1.900	3.471 $\pm$ 3.061	0.8	9.453 $\pm$ 799	2.7
15	LL	1	5	15.565 $\pm$ 1.940	2.705 $\pm$ 666	5.8	2.297 $\pm$ 594	2.062 $\pm$ 1.824	1.1	18.977 $\pm$ 1.326	9.2
16	LL	2.8	12	62.315 $\pm$ 7.277	13.185 $\pm$ 5.028	4.7	7.386 $\pm$ 2.416	3.833 $\pm$ 801	1.9	9.881 $\pm$ 2.245	2.6
17	LL	0	18	24.536 $\pm$ 6.803	9.266 $\pm$ 3.191	2.6	4.687 $\pm$ 242	8.574 $\pm$ 2.544	0.5	23.482 $\pm$ 123	2.7
c.p.m. mean $\pm$ s.e.				40.650 $\pm$ 7.967	5.944 $\pm$ 1.007		7.250 $\pm$ 2.529	6.097 $\pm$ 1.328		18.326 $\pm$ 1.625	
SI mean $\pm$ s.e.						8.95 $\pm$ 2.28			1.13 $\pm$ 0.15		5.41 $\pm$ 1.47

PBMC were prepared from defibrinated venous blood by centrifugation on Ficoll-Isopaque. 0.2×10^6 cells were cultured in triplicate in 96-well round-bottomed trays (Linbro) in 200 μ l of RPMI 1640 medium containing 20% pooled, heat inactivated, normal human serum, 2 mmol l⁻¹ L-glutamine and antibiotics. Test cultures contained 25 μ l of whole washed *M. leprae* with a concentration of 3×10^7 bacilli per ml, which gives optimal proliferation in tubercloid patients or PPD (25 μ l of 100 μ g ml⁻¹). To the cultures were added 100 μ l per well of T-cell conditioned media. The conditioned media used were Lymfocult T (Biotest)²³ or preparations made by ourselves by similar procedures²³. Supernatants were collected after incubation for 48 h (RPMI 1640 + 1% inactivated normal human serum) and stored frozen until used. The data presented are based on Lymphocult T used in a dilution 1:100. Dilution $\geq 1:50$ was found to be non-mitogenic on normal PBMC (day 3). This dilution was found to be analogous in IL-2 activity as compared with antigen-stimulated (for example, BCG) normal PBMC. Appropriate control cultures were set up containing conditioned media but no antigen. Cultures were maintained at 37 °C in 5% CO₂-humid air. For measurement of DNA synthesis the cells were exposed to 1 μ Ci ³H-thymidine per well on day 5 and collected 20 h later with multiple cell culture collector (Skatron). ³H-dThd incorporation was determined by scintillation counting (LKB). The patients were selected from the All Africa Leprosy and Rehabilitation Training Centre (ALERT) at Addis Ababa. The patients were classified by histopathological criteria according to the Ridley-Jopling scale²⁴. All had skin biopsy taken during active disease to support clinical classification. All patients were lepromin negative by the Mitsuda test. Stimulation index = c.p.m. in cultures with *M. leprae*/c.p.m. in cultures without *M. leprae* or PPD/without PPD.

For column 1 versus column 2; $P = 0.0003$ (Wilcoxon matched pairs, two tailed).

For column 1 versus column 3; $P = 0.002$ (Wilcoxon, matched pairs, two tailed).

* Chemotherapy with dapsone or dapsone + rifampicin.

Table 2 IL-2 activity in *M. leprae*-stimulated PBMC supernatants from lepromatous patients and healthy contacts

Conc. of supernatant added	c.p.m. $\pm$ s.d.		Medium
	1:2	1:4	
Healthy contact number 1	25.289 $\pm$ 10.443	13.998 $\pm$ 9.898	1.342 $\pm$ 852
Healthy contact number 2	25.601 $\pm$ 6.007	32.412 $\pm$ 560	1.952 $\pm$ 264
Healthy contact number 3	12.601 $\pm$ 139	10.049 $\pm$ 3.401	2.352 $\pm$ 169
Healthy contact number 4	19.096 $\pm$ 4.300	20.541 $\pm$ 5.011	1.841 $\pm$ 264
Patient number 5	2.033 $\pm$ 1.093	2.783 $\pm$ 1.392	2.964 $\pm$ 1.489
Patient number 6	2.268 $\pm$ 79	3.784 $\pm$ 2.441	2.453 $\pm$ 67
Patient number 12	551 $\pm$ 390	617 $\pm$ 621	1.067 $\pm$ 458
Patient number 13	1.971 $\pm$ 197	2.981 $\pm$ 677	1.401 $\pm$ 551
Patient number 15	2.332 $\pm$ 743	2.148 $\pm$ 1.200	2.406 $\pm$ 1.993

Patient no. indicates patient no. in Table 1 (all LL). Healthy contacts were four *M. leprae*-sensitive subjects working with leprosy patients at ALERT. The tests on patients and healthy contacts were carried out simultaneously. For *M. leprae* driven IL-2 production, 5×10^6 PBMC were incubated in 2 ml culture medium in the presence of 2×10^6 *M. leprae* bacilli in round-bottomed plastic tubes at 37 °C for 48 h. The supernatants were collected, sterile filtered, and assayed in a micromethod for IL-2 activity²⁵. Briefly, T cells (5×10^4) obtained from a PHA-initiated T-cell line were incubated with two dilutions (1:2 and 1:4) of the supernatant in flat-bottomed microtitre plates for 48 h. Cell proliferation was assessed by adding 1 μ Ci of ³H-thymidine per well during the final 12 h of culture.

Table 3 Effect of TCM and *M. leprae* on DNA synthesis of PBMC cultures from individuals not exposed to *M. leprae*

T-cell conditioned medium <i>M. leprae</i>	³ H-thymidine incorporation (c.p.m. $\pm$ s.d.)					
	+	+	-	+	-	-
	1	2	SI	3	4	SI
Subject no.						
1	14.466 $\pm$ 1.187	15.084 $\pm$ 1.346	1.0	1.577 $\pm$ 1.160	1.699 $\pm$ 1.364	0.9
2	13.060 $\pm$ 2.158	14.282 $\pm$ 2.408	0.9	1.113 $\pm$ 329	896 $\pm$ 289	1.2
3	12.062 $\pm$ 3.458	9.078 $\pm$ 1.587	1.3	808 $\pm$ 114	594 $\pm$ 60	1.4
4	7.734 $\pm$ 537	7.991 $\pm$ 1.163	1.0	234 $\pm$ 41	292 $\pm$ 141	0.8
Mean $\pm$ s.e.	11.830 $\pm$ 1.451	11.608 $\pm$ 1.796	1.05 $\pm$ 0.19	933 $\pm$ 281	870 $\pm$ 302	1.08 $\pm$ 0.14

PBMC of Norwegian blood donors were separated, cultured and collected as described in Table 1 legend. The same batch of TCM was used for data shown in Tables 1 and 3.

in lepromatous PBMC was not simply due to an elevation of background responses to *M. leprae* found in normal human PBMC. Compared with the patient group, the background (that is, column 4, Tables 1, 3) was very low in the control group. On the other hand, TCM induced, not unexpectedly, DNA synthesis on day 5 in healthy individuals, because trace amounts of mitogen + IL-2 should be enough to stimulate a few T cells, but this was only seen occasionally in the patients 5, 13 and 16. The reason for these differences remains unclear. They appear in part to be due to the uncommon pattern found in patient 8 and 9 with very high background and much lower counts with TCM. Moreover, technical differences cannot be ruled out, as these studies were carried out in two different laboratories. Among other possibilities not only disease-specific phenomena, for example, a disseminated mycobacterial infection affecting lymph node structure and presence of immune complexes³ have to be considered but also differences in socioeconomics, for example nutrition and continuous exposure to parasites.

The phenotype of the proliferating cell of lepromatous patients exposed to TCM + *M. leprae* was studied with OKT4 and OKT8 monoclonal antibodies (Ortho), as lepromatous patients have been found to have increased numbers of activated OKT8⁺ cells among PBMC⁹⁻¹¹. By indirect fluorescent staining¹² the majority of cells on day 5 were found to have OKT4⁺ features (78 ± 2.61 OKT4⁺, 19.1 ± 2.66 OKT8⁺, $n = 6$ LL). Since this subset comprises cells carrying inducer/helper functions¹³, our findings are encouraging for the eventual aim of restoring immunological competence in such patients.

Together our findings suggest that the proliferative defect to *M. leprae* of lepromatous T cells is not because of the absence of or a generalized lack of triggering of such cells but commonly due to a deficiency in events leading to antigen-nonspecific factor production. The factor most likely to be involved in the restorative effect observed, appears to be IL-2. We have failed to find a restorative effect with IL-1 (data not shown), but since crude TCM was used, a role of other lymphokines cannot be ruled out. Restorative effects *in vitro* of IL-2 have previously been reported in primary immunodeficiencies¹⁴ in aged immunodeficient mice¹⁵ and rats¹⁶. To our knowledge this is the first report of a restorative effect of IL-2 or related factors in a disease-associated state of specific immunological unresponsiveness. The mechanism behind the deficiency in T-cell factor production remains unclear. It may be related to the presence of suppressor cells in such patients^{11-13, 17-19}. In experimental systems, T-suppressor cells²⁰ and T-cell derived suppressor factors²¹ have been described which suppress IL-2 production. The OKT8⁺ suppressor cell described by Mehra *et al.*¹¹⁻¹³ in lepromatous patients may be a good candidate for suppression of IL-2 production, although suppression of T-cell proliferation does not appear to be restricted to this phenotype²².

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Control of Ca²⁺ in rod outer segment disks by light and cyclic GMP

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Photons absorbed in vertebrate rods and cones probably cause electrochemical changes at the photoreceptor plasma membrane by changing the cytoplasmic concentration of a diffusible transmitter substance, reducing the Na⁺ current flowing into the outer segment of the cell in the dark, to produce the observed membrane hyperpolarization that is the initial excitatory response¹⁻⁴. Cyclic GMP has been proposed as the transmitter because a light-activated cyclic GMP phosphodiesterase (PDE) has been found in rod disk membranes^{5,6} and because intracellularly injected cyclic GMP⁷ reduces rod membrane potentials. Free Ca²⁺ has also been proposed because increasing external [Ca²⁺] quickly and reversibly reduces the dark current and divalent cationophores increase the Ca²⁺ sensitivity⁸. Ca²⁺ efflux from rod outer segments (ROS) of intact retinas occurs simultaneously with light responses^{9,10}. Vesicles prepared from ROS disk membranes become more permeable on illumination, releasing trapped ions or molecules¹¹⁻¹³, but intact outer segment disks have not previously been found to store sufficient Ca²⁺ in darkness and to release enough in light to meet the theoretical requirements for control of the dark current by varying cytoplasmic Ca²⁺ (refs 14-18). We now report experiments that show the required Ca²⁺ storage and release from rod disk membranes suspended in media containing high-energy phosphate esters and electrolytes approximating the cytoplasmic composition of live rod cells. Cyclic GMP stimulates Ca²⁺ uptake by ROS disks in such media.

Concentrated suspensions of ROS disks together with some nuclei, mitochondria and other subcellular fragments were prepared as described previously¹⁹. ATP, GTP and creatine phosphate were added to approximate cytoplasmic levels to form an artificial cytoplasm 'medium P' (Fig. 1) whose initial Ca²⁺ activity was <50 μM. Free Ca²⁺ activity was measured with Ca-sensitive electrodes⁹ and uptake of added ⁴⁵Ca²⁺ into ROS disks and other particles was measured by separating particles from the suspending medium P by centrifugal separation through silicon oil of density 1.04 (ref. 19). By spreading thin layers of undiluted ⁴⁵Ca²⁺-labelled suspension between polyester films and freeze-drying the layers, the accumulation of exchangeable Ca²⁺ by the various particles was studied²⁰.

Ca²⁺ electrodes showed that the suspensions of ROS in medium P absorbed Ca²⁺ from the medium in the dark. A_{Ca} in the suspension began at 5-15 μM (1-5 min after isolation), falling after 15-20 min to 2-3 μM where it remained for several hours. The lowest A_{Ca} levels reached varied little between experiments, but the initial value depended on how thoroughly Ca²⁺ was washed from the retinas before milling. As A_{Ca} was usually slowly rising or falling in the suspension, effects of light and of injected substances were corrected for drift by subtracting a linear best fit to the sloping baseline measured just before the illumination or injection.

Dim light produced an increase in A_{Ca} in the suspensions. In steady light (1.5 photoisomerizations per disk per s) the rise

was sigmoidal, lasted longer than the illumination and often raised A_{Ca} by $>0.5 \mu M$ (Fig. 1A). 2-s Flashes caused a prompt initial rise usually followed after 1 min by a larger slow increase. The sizes of both phases increased with exposure (Fig. 1B) up to at least 1 photoisomerization per disk. The initial rise was limited by the exponential response lag of the Ca-sensitive electrode.

To estimate the Ca^{2+} release underlying the observed transients, 1–10 μl of 50 μM Ca^{2+} was injected into the ROS suspension and the peak size of the resulting A_{Ca} increase noted (see Fig. 3B). The ratio 1/B of this increase to that seen when a similar injection was made into the same volume of particle and nucleotide-free medium P was always 3–10, because the phosphates present bind Ca^{2+} reversibly and the particles take it up. If these two processes are considered as Ca^{2+} buffering, the rate of change in A_{Ca} in a light response can be converted to an equivalent light-induced influx j_{Ca} into the free Ca^{2+} pool by the relation

$$j_{Ca} = BV \, dA_{Ca}/dt \quad (1)$$

where V is the suspension volume.

Table 1 shows integrated Ca^{2+} effluxes for the first 90 s after different light exposures in aliquots from three similar ROS suspensions.

Although the results varied between trials, the total Ca^{2+} released per ROS disk having ≥ 1 photoisomerization varied less than eightfold over an exposure range of 3,000. However, the responses are too variable to establish whether a single photoisomerization can discharge Ca^{2+} in more than one disk.

Total Ca^{2+} efflux from the outer segments during the first 90 s after exposures of 10^6 photoisomerizations per disk was 0.05–0.2 Ca^{2+} per rhodopsin chromophore in the particles, and the light-sensitive Ca^{2+} store was completely discharged. No light-induced pH changes were seen (0.001 pH unit detectable) by miniature glass electrode. In simultaneous recordings from two aliquots of ROS suspension one of which was illuminated at a time, A_{Ca} rose in the exposed sample but not the dark control.

Is Ca^{2+} associated with particles in the suspension or with soluble binding sites? The oil filtration method showed that ^{45}Ca added to a ROS suspension 5 min after isolation penetrated the particles with half time of 5–15 min (Fig. 2, triangles). Light caused some ^{45}Ca to shift from the particles to the aqueous phase. In ROS suspensions containing mitochondria, 3–8% of exchangeable particle Ca^{2+} was released by light within 1 min. The addition of cyclic GMP to an initial concentration of 500 μM caused particle Ca^{2+} to increase and the light-releasable fraction to rise to 10–20%, suggesting that cyclic GMP expands the light-sensitive Ca store or increases its Ca turnover²¹.

Cyclic GMP alone does not drive Ca^{2+} uptake by particles. If ATP or GTP and phosphocreatine were not provided or if the NTP:NDP ratio fell below 1.0 during an experiment, particles lost Ca^{2+} , the Ca^{2+} light response disappeared, and cyclic GMP additions became ineffective. A light-induced fall in ATP

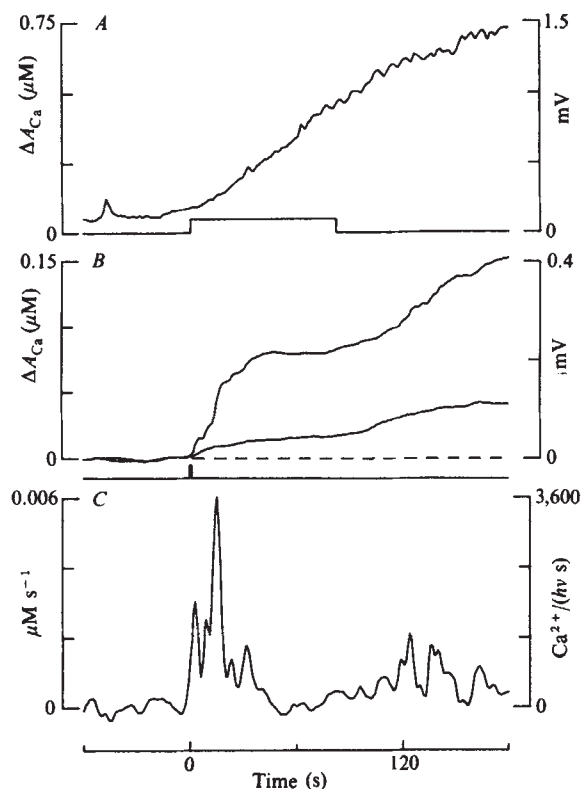


Fig. 1 Light-induced rise of A_{Ca} in 100 μl of medium P containing ROS measured with a Ca^{2+} -sensitive electrode. **A**, Continuous illumination, 1.5 $h\nu$ -absorbed per disk per s. **B**, 2 s of illumination delivering 0.15 or 0.015 $h\nu$ -absorbed per disk. Curves were corrected for a small drift in the baseline. Total Ca^{2+} efflux from ROS, calibrated by Ca^{2+} injection as in Fig. 3B, was equivalent to 1 Ca^{2+} per 20 rhodopsin molecules. Buffering power of particle suspension for Ca^{2+} was 9.5 times that of medium P with no added phosphates. **C**, Numerical differentiation of 0.15 $h\nu$ per disk response from **B**. Left scale, rate of change of A_{Ca} with time. Right scale, net Ca^{2+} efflux from particles, corrected for $9.5 \times Ca$ buffering of suspension.

Methods: Frogs (*Rana catesbiana*) were dark adapted for 8–12 h and their retinas removed in oxygenated amphibian Ringer containing glucose. Only IR illumination ($\lambda > 800$ nm) was used during and after dissection. After a 10–20-s rinse in a Ringer containing $<10 \mu M$ Ca^{2+} , two to six retinas were milled, then filtered¹⁹, in an intracellular medium (medium P) (NaCl, 10–40 mM; K isethionate, 100–70 mM; K_2HPO_4 , 1 mM; PIPES, 10 mM, pH 6.8 containing phosphocreatine (PCr), 10–20 mM; ATP and GTP, 5 mM each; and $MgSO_4$, 16 mM). No Ca^{2+} was added, but enough was present in the Na and K salts to yield Ca^{2+} activities of 10–50 μM . A 1-min spin at 35 g pelleted intact ROS, leaving most free mitochondria in the supernatant, which was discarded. The ROS were resuspended in medium P and centrifuged again at 16,000g for 1 min to break their plasma membranes, making them leaky to nucleotides, Ca^{2+} and didansyl cystine (DDC). The resuspended ROS had $>95\%$ leaky plasma membranes, as judged by DDC fluorescence microscopy³⁵, along with nuclei and some mitochondria. Rhodopsin concentrations were measured in the suspensions by extracting aliquots with 1% buffered cetyltrimethylammonium chloride and bleaching the extracts in a spectrophotometer in 1% hydroxylamine at pH 7.1. Counts of numbers and lengths of ROS in the suspensions yielded 2×10^6 rhodopsin chromophores per rod disk if the spacing of ROS was taken to be 25 nm. In all suspensions rhodopsin concentrations were 10–40 μM . Free Ca^{2+} activity (A_{Ca}) was measured in 20–200 μl of ROS suspension in conical plastic test tubes stirred by continuous rotation. Small Ca^{2+} -sensing electrodes made from glass capillaries with silicone-treated tips containing the ionophore ETH 1001 (10%) were used^{36,37}. Response time constants were 3–20 s at $A_{Ca} < 5 \mu M$. A Teflon plug was placed around the electrodes to prevent evaporation. Aliquots (1–10 μl) of Ca^{2+} (50 μM) or cyclic GMP (5 mM) solutions were injected and samples were withdrawn for nucleotide analysis by HPLC or radioimmunoassay (RIA) with microlitre syringes. Rhodopsin concentration was 25 μM .

Table 1 Ca^{2+} ions released in 90 s from ROS by light flashes

Mean photoisomerizations per disk	Ca^{2+} per photoisomerization	Ca^{2+} per disk having 1 or more photoisomerization	n
0.15	$28,000 \pm 15,600$	$4,190 \pm 2,333$	2
0.4	$4,370 \pm 1,060$	$1,750 \pm 420$	3
4	490 ± 20	$1,960 \pm 60$	3
40	86 ± 28	$3,460 \pm 1,110$	7
400	30 ± 18	$12,100 \pm 5,300$	2

Values are combined data from three ROS suspensions with rhodopsin concentrations of 20, 20 and 24 μM , given as the mean $\pm$ s.e. of estimate. Release is expressed as equivalent number of Ca^{2+} ions required to be injected into ROS suspension as 50 μM Ca^{2+} to produce a rise in A_{Ca} at 90 s identical to that caused by light flashes with photoisomerizing effects shown in the left column.

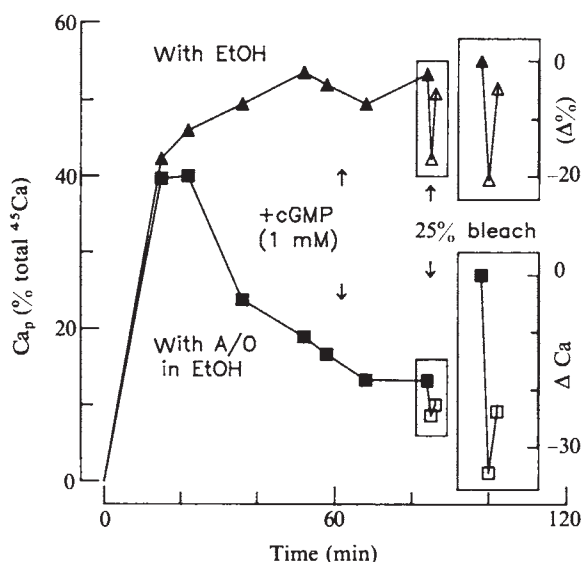


Fig. 2 ^{45}Ca exchange with particles in ROS suspensions in medium P with and without suppressors of mitochondrial Ca^{2+} uptake. Ca movements in the particulate component of ROS suspensions were studied by incubating 1-ml portions with 1–10 μCi of added ^{45}Ca . 50- μl aliquots were layered on silicone oil (specific gravity 1.040), in 1.5-ml centrifuge tubes. Some aliquots were exposed to 1-ms light flashes. After a timed delay of 1–2 min illuminated and dark controls were spun together at 16,000g for 60 s, transferring the particles in <4 s down from the upper aqueous phase through the silicone oil into 10- μl volume of 10% w/v glucose in 0.5 M HNO_3 at the bottom of the tubes¹⁹. The tubes were placed in ethanol/dry ice slurry and the frozen upper aqueous and lower extractant phases collected separately and counted. Tritiated water (5 μCi), added to the suspensions in a few experiments, indicated that about $3\times$ the ROS volume (<1% of the upper phase volume) but 5–60% of the total upper phase ^{45}Ca transferred through the oil with the particles. Tracer added at $t=0$ raised A_{Ca} by <0.5 μM . Cyclic GMP added at $t=60$ min increased particle Ca^{2+} (Ca_p) slightly. Flashes bleaching 25% of rhodopsin at $t=80$ min released 20–30% of Ca_p (triangles). If 10 μM antimycin A and 10 μM oligomycin (A/O) were added in ethanol (squares) (final concentration 1%), less Ca_p was retained, but light released >30% (inset). Ca_p lost in A/O was presumably in mitochondria and was light insensitive.

or GTP was not necessary for the observed Ca^{2+} light response, however, because light responses were obtained when NTD:NDP ratios were stabilized at >4.0 by 20 mM of added PCr. Either added GTP or ATP alone supported the Ca^{2+} uptake and light response mechanisms but because low concentrations of both nucleotides were carried with ROS during isolation and the suspension contained mechanisms for phosphate transfer between the A and G systems and from phosphocreatine, the immediate energy source for Ca^{2+} uptake is uncertain.

Antimycin (10 μM) plus oligomycin (10 μM) (A/O) caused 75–90% of particle ^{45}Ca to be lost but the light-releasable fraction of that remaining rose to 25–50% (Fig. 2, squares). Ruthenium red (10 μM) (RR), which inhibits mitochondrial Ca uptake²², acted similarly.

Cyclic GMP had a marked effect on Ca^{2+} in ROS suspensions when added to them. The addition of medium P of $A_{\text{Ca}} \sim 10 \mu\text{M}$ to a ROS suspension caused an initial jump in A_{Ca} because that of the suspension was lower (Fig. 3B). The added Ca^{2+} was absorbed and A_{Ca} returned to the preinjection baseline within 4–8 min. Injection of cyclic GMP in medium P always increased Ca^{2+} uptake, usually driving A_{Ca} well below the levels observed after injections of Ca^{2+} alone (Fig. 3A). If A_{Ca} was $\leq 2 \mu\text{M}$, a cyclic GMP injection did not drive A_{Ca} below the preinjection level, but the half time for uptake of the added Ca^{2+} was less than with medium P alone. HPLC showed that the suspension hydrolysed the added cyclic GMP in 5–20 min, as reported previously²³. At the end of the rapid Ca^{2+} uptake

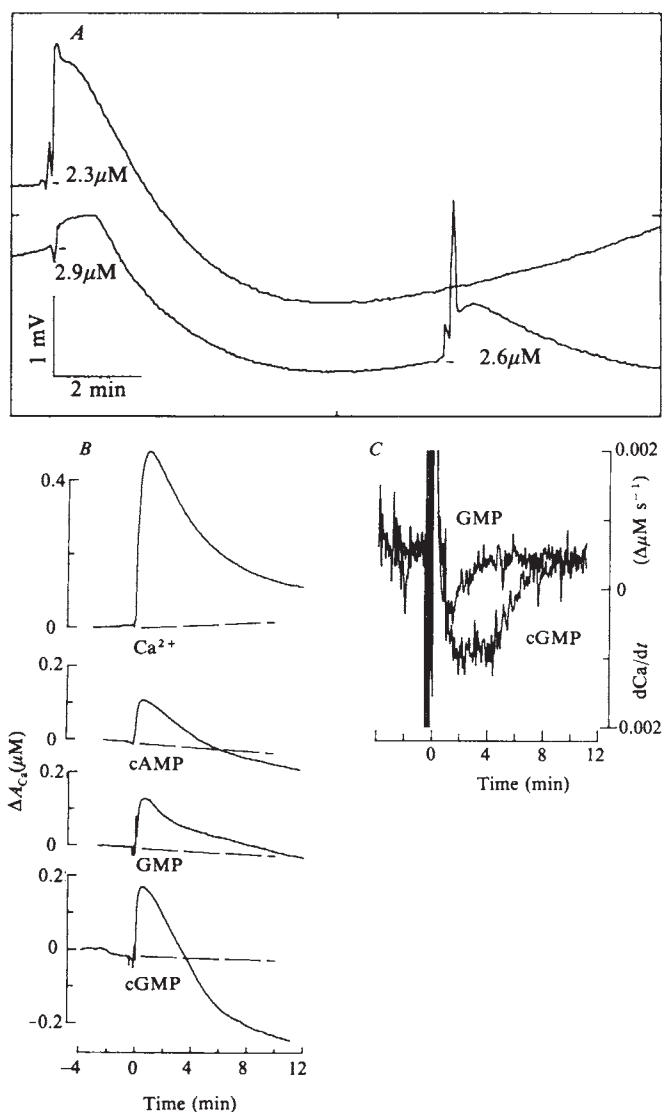


Fig. 3 Effect on A_{Ca} of adding Ca^{2+} + cyclic GMP or Ca^{2+} alone to ROS suspensions in NTP medium. Initial rhodopsin concentration was 30 μM before injections. A, Ca^{2+} plus cyclic GMP injected. Initial [cyclic GMP], 0.5 mM. The lower record was taken 1 h after the upper. Ca^{2+} taken up was proportional to ambient A_{Ca} . The second cyclic GMP addition on the lower trace stimulated less Ca^{2+} uptake than the first. B, Injections of medium P plus 50 μM Ca^{2+} compared with Ca^{2+} plus GMP or cyclic GMP solution (final concentrations, 0.5 mM in medium P). Initial suspension volume, $\sim 100 \mu\text{l}$. 50 μM Ca^{2+} standard was prepared in a similar medium without nucleotides. 10- μl injections were performed on two suspensions in parallel, with the order of cyclic GMP or control addition varied. Similar responses to test injections were obtained on each channel. No substance tested (phosphocreatine, GMP, cyclic AMP, taurine or isobutyl methylxanthine in medium P) stimulated Ca^{2+} removal as did cyclic GMP. C, Numerical derivatives of records from B showing removal of Ca^{2+} following injections of GMP or cyclic GMP. Cyclic GMP increased both the maximum rate and duration of Ca^{2+} uptake following the addition.

phase, when A_{Ca} levels had returned to the preinjection level, HPLC showed that cyclic GMP had fallen to the previous <10 μM level typical of ROS suspensions, much lower than that in intact rods^{16,24–27}. Injections that raised [cyclic GMP] to >2 mM produced longer periods of uptake but also added more Ca^{2+} with the increased injected fluid volume, so that the most striking Ca^{2+} uptake was observed with the smallest Ca^{2+} -cyclic GMP additions. Cyclic GMP alone did not stimulate Ca^{2+} if ATP or GTP were absent.

Which particles in the suspension accumulate ^{45}Ca ? In medium P much of the particle $^{45}\text{Ca}^{2+}$ was apparently in

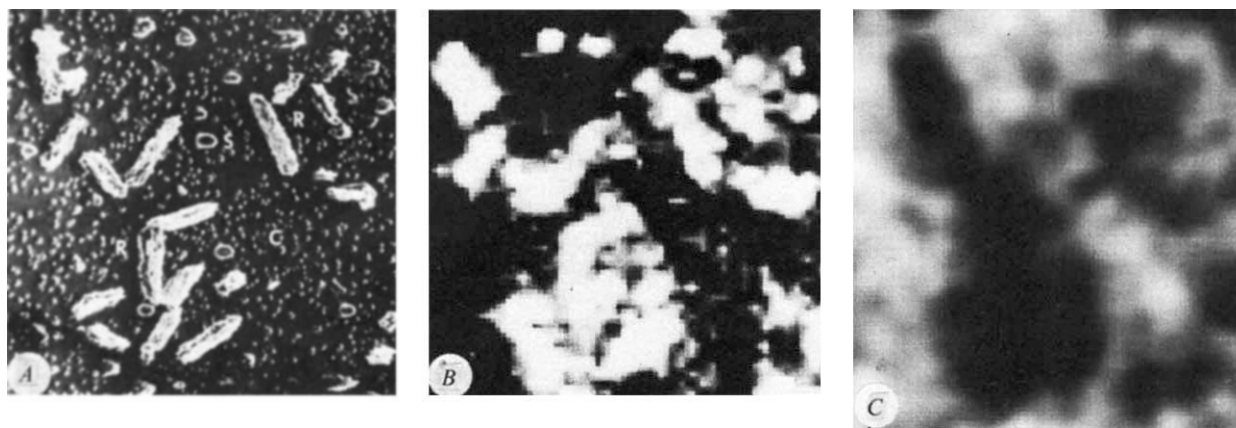


Fig. 4 Potassium and ^{45}Ca distributions in freeze-dried layers of undiluted dark-adapted ROS suspensions on 2 μm polyester film. **A**, Secondary electron image in scanning electron microscope: R, rod outer segment; S, glass microsphere; C, crystals of potassium isethionate plus phosphocreatine. **B**, Distribution of silver $L\alpha$ emission in autoradiographic emulsion on the reverse side of the polyester film opposite the area shown in **A**. **C**, Distribution of potassium $K\alpha$ emission from the region shown in **A**, scanned with 10 kV, 2.5 nA electron beam.

Methods: See ref. 20 for the method used. Picture widths, 210 μm . ROS suspensions were incubated for 30 min with $10 \mu\text{Ci ml}^{-1}$ of ^{45}Ca + 1 mM cyclic GMP in medium P containing 10 μM ruthenium red to suppress mitochondrial Ca^{2+} accumulation. Aliquots (1.5 μl) were spread without dilution between tightly stretched 2- μm thick polyester films, and 4- μm glass microspheres were added to act as spacers. The assembly was frozen in liquid N_2 , and freeze-dried as described elsewhere²⁰. An Ilford K5 autoradiographic emulsion was spread on the reverse side of the film freeze-dried suspension, exposed for 4 days and developed. Both sides of the film were coated with 50 nm of evaporated carbon. Corresponding regions of each side of the film were scanned with the electron beam of a scanning electron microscope and two-dimensional maps of characteristic X-ray emission from S, P, Cl and K (particle side) and Ag (emulsion side) made by energy-dispersive X-ray spectroscopy. After image processing by computer to correct for the X-ray continuum under each characteristic elemental emission line and for nonlinearity in the response of the autoradiographic emulsion to ^{45}Ca β radiation, the maps were compared to locate exchangeable Ca^{2+} among the particles and dried residue of medium P. Rhodopsin concentration in ROS suspension was 40 μM . In **B**, the image was reversed and numerically processed to give highlight intensities proportional to the ^{45}Ca radiation intensity in the source. The diffusion of Ag images is due to divergence of ^{45}Ca β radiation in the 2 μm thickness of the support and the 3 μm thickness of the Ilford K5 autoradiographic emulsion. Both ROS and glass spheres show ^{45}Ca binding. 10 kV electron beam, 2.5 nA. Analysis of autoradiographic images indicated that ROS contained at least 20 $\times$ as much ^{45}Ca as did an equivalent volume of the suspending medium. Simultaneous measurement of particle $^{45}\text{Ca}^{2+}$ from the same suspension by the method of Fig. 2 confirmed that the particles contained >15% of total ^{45}Ca . Picture resolution, 50 $\times$ 50 pixels. In **C**, light areas denoting high K abundance correspond to the distribution of small K isethionate crystals, not ^{45}Ca . The image has been convolved with the 6.8 μm diffusion function of the autoradiographic process so as to match the resolution in **B**. Most of the potassium was found in the dried medium between the particles, while most of the ^{45}Ca was in ROS.

mitochondria, even when most free mitochondria in the suspension were removed by differential sedimentation. >75% of this particle, Ca^{2+} was released by RR or A/O, and >90% by ionophores. Suspensions of retinal mitochondria alone took up added Ca^{2+} but did not lower steady-state A_{Ca} below 3–5 μM . Cyclic GMP did not stimulate Ca^{2+} uptake by mitochondria nor did they release Ca^{2+} in light. However, ROS disks also take up Ca^{2+} , because RR or A/O never completely discharged particle Ca^{2+} , and its uptake was observed in 10 μM RR, which completely inhibits mitochondrial Ca^{2+} uptake²² (data not shown).

^{45}Ca radioautographic images were associated with the isolated outer segments suspended in medium P containing 10 μM RR (Fig. 4). $^{45}\text{Ca}^{2+}$ in ROS, estimated from the intensity of the silver images, was enriched 20-fold relative to the suspending medium. This value agreed with measurements on the same suspension by the oil spin method and was enough to account for the observed light release. Little ^{45}Ca was seen near mitochondria in such preparations, but if RR was omitted, most of the tracer was located over a few intensely radioactive spots that were apparently mitochondria. Little radioactivity was seen over ROS in such preparations. Evidently, ROS store most of the exchangeable and light-releasable $^{45}\text{Ca}^{2+}$, if mitochondrial Ca^{2+} accumulation is suppressed.

The Ca^{2+} model for vertebrate visual transduction has two independent parts. According to the control hypothesis, the dark current is modulated by the cytoplasmic Ca^{2+} level, which rises in light. The storage hypothesis originally supposed that Ca^{2+} is accumulated in ROS disks by active uptake and released in light by passive diffusion down a thermodynamic gradient into the cytoplasm. Alternatively, cytoplasmic Ca^{2+} could also rise if light released Ca^{2+} from binding sites or if some Ca^{2+} uptake mechanism were light-inhibited.

If dark current control by light operates by changing Ca^{2+} storage, the store must have three properties. (1) Each unit (presumably a disk) must release enough Ca^{2+} to change the average cytoplasmic level in the outer segment by at least 3% per absorbed photon (or 5% per photoisomerization (see ref. 28)) within the 0.1–1 s onset phase of single photon responses²⁹. (2) The Ca^{2+} released from an excited disk must not be absorbed by the store in the neighbouring unexcited disks during the onset of a photon response. Otherwise the uptake mechanism in the unexcited disks would buffer the cytoplasmic Ca^{2+} level so strongly that single photon responses could not occur (manuscript in preparation). (3) The cycle of release and reaccumulation of Ca^{2+} must expend free energy.

The present experiments and previous ones⁹ suggest that ROS contain a Ca^{2+} store that at least meets requirements (1) and (3) and probably requirement (2). More than 10,000 ions were rapidly released into the photoreceptor cytoplasmic space per absorbed photon in some trials, although the Ca^{2+} flux from isolated ROS disks often lasted longer than electrical responses of intact retinas. A longer response might result if the mechanism that terminates the efflux were slowed by dilution of soluble components. The ROS take up Ca^{2+} in the dark, a requirement to resensitize the cell though probably not essential to allow recovery of the dark current, as Ca^{2+} is so rapidly extruded by live rods⁹. But the uptake is slow enough for it not to compete with the light-induced rise in A_{Ca} . The NTP requirement for Ca^{2+} uptake and retention suggests, but does not prove, that Ca^{2+} is sequestered in the disks by active uptake.

Ca^{2+} quickly suppresses photoreceptor plasma membrane Na^+ conductance, but apparently not by changing cytoplasmic cyclic GMP^{26,30}. Although cyclic GMP affects ion fluxes from disk membrane vesicles³¹, most observed effects of raising photoreceptor cyclic GMP might be explained as effects on

Ca^{2+} metabolism³². Our results suggest how. By stimulating transfer of cytoplasmic Ca^{2+} to the light-sensitive Ca^{2+} store, cyclic GMP could affect the state of photoreceptor adaptation. Although the light-induced decrease in photoreceptor cyclic GMP, measured in physiological conditions, is small and slow^{26,33,34}, such a change would tend to reduce stored Ca^{2+} and thus decrease the amount available for release by absorbed photons.

A preliminary account of this work has been presented elsewhere²⁷. We thank Margaret Foster for microprobe analyses, Benes Trus for image processing and Shuko Yoshikami for helpful advice.

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Strong homology in promoter and 3'-untranslated regions of chick and rat α -actin genes

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We report here a comparison of the nucleotide sequences of the chick and rat skeletal muscle α -actin genes which reveals strongly conserved blocks of sequence in the putative transcriptional promoter and 3'-untranslated regions. This is the first instance of strong sequence conservation in untranslated gene regions between birds and mammals, other than the CAAT and ATA promoter homologies and the putative poly(A) addition signal, AATAAA¹. The strong conservation of these sequences suggests that they may have a role in the tissue-specific expression of the skeletal muscle α -actin gene.

Figure 1a shows the general structure of the chick skeletal muscle α -actin gene determined from its nucleotide sequence². It consists of seven exons (numbered I–VII in Fig. 1a) the first of which is a short untranslated region and the last encodes the C-terminal 47 amino acids plus a 272-nucleotide 3'-untranslated region. The sequence of the rat skeletal muscle α -actin gene³ demonstrates that its structure is identical with respect to intron position and is similar with respect to the length of the untranslated regions in exons I and VII. Only partial homology of intron position is seen for β -actin genes and for sea urchin actin genes (for review see refs 2, 3). Figure 1a also shows the positions of three sequence blocks (A–C) that are conserved between the skeletal muscle α -actin genes of chick and rat. Figure 1b gives the relevant nucleotide sequences.

Region A is a 20-nucleotide block beginning 95 nucleotides upstream from the site of transcriptional initiation in the chick (100 nucleotides upstream in the rat). Over this 20-nucleotide stretch there is only a single nucleotide difference between chick and rat. Outside this block of homology there is relatively low homology even for the untranslated first exon (see below). This homology block contains the sequence CAAAT localized at position –85 (chick) which is the putative CAAT homology found in most eukaryotic genes 80–100 nucleotides upstream from the point of transcriptional initiation¹. Homology regions B and C are found within the 3'-untranslated regions of the gene. The first of these (B in Fig. 1a, b) is a 14-nucleotide stretch beginning 32 nucleotides downstream from the translation termination codon both in the chick and rat. Over this stretch there is only a single nucleotide difference between the two genes. Analysis of the published sequence of a human cardiac α -actin gene, which is closely related to the skeletal muscle α -actin gene, shows a match for the first 10 nucleotides of this sequence in the identical location⁴. In addition, a partial match for this sequence (12 of 14 nucleotides) is found at an undetermined location in the 3'-untranslated region of a rat myosin light chain cDNA⁵. Homology region C (Fig. 1a, b) spans the 86 nucleotides preceeding the putative poly(A) addition signal, ATTAAA. Over this 86-nucleotide stretch there are 18 changes or deletions in comparing rat to chick; 11 of these changes occur in three clusters leaving stretches of perfect homology up to 19 nucleotides long. Although this degree of sequence conservation is high it is important to note that some of the high homology is attributable to A + T-rich stretches.

Does the strong sequence conservation reported here reflect selection of functionally important regions or an unusual property of the skeletal muscle α -actin gene locus? Actin genes are unusual in that their protein-coding regions are strongly conserved over vast evolutionary distances^{2,4}. However, the conserved regions noted here between the chick and rat skeletal muscle α -actin genes have discrete boundaries and are localized in precise relationship to the CAAT homology (region A), the termination of translation (region B) and the putative poly(A) addition signal (region C). Other regions outside these homology blocks are not well conserved. For example, the 5'-untranslated exons of the two genes show relatively low homology (Fig. 1c) as do the 3'-untranslated regions between B and C (not shown). We conclude, therefore, that strong sequence conservation is not a uniform property of the skeletal muscle α -actin gene locus and that the localized conservation reported here is probably functional and maintained by selection pressure. While experiments using mutant genes will be necessary to determine the function of these conserved regions their location suggests that each may have a different role. For example, the presence of the CAAT homology within region A suggests that this sequence may be involved in transcriptional initiation. The location of homology regions B and C within the 3'-untranslated region is consistent with their functioning in a post-transcriptional capacity such as poly(A) addition or translation. As these sequence elements have not been found in several other eukaryotic genes^{6–18} it is possible that they are unique to genes expressed in the muscle phenotype. Support for this conclusion also comes from the fact that these sequence



Fig. 1 *a*, Organization of the chick skeletal muscle α -actin gene and the positions of sequence homology with the rat skeletal muscle α -actin gene. The size and positions of the seven exons and six introns were determined by nucleotide sequencing of the entire gene² and are drawn to scale. The structure of the rat skeletal muscle α -actin gene is essentially identical except for the relative sizes of the introns³. Thin horizontal lines designate flanking and intron DNA; boxes designate transcribed regions of the gene. The solid and open boxes represent untranslated and translated regions of the mature mRNA, respectively. A–C designate the positions of sequences found to be homologous with the rat gene (sequence C is shown in two parts for convenience). *b* Shows the nucleotide sequences of these homology blocks. The sequences labelled (1), (2) and (3) are taken from the published sequences of the chick skeletal muscle α -actin gene², the rat skeletal muscle α -actin gene³, and the human cardiac α -actin gene⁴, respectively. Matched nucleotides are designated by double dots; deletions are denoted by a dash. The putative CAAT homology in A and ATAAA homology in C are indicated by asterisks. The precise positions of each homology block are given in the text. In *c* the sequence for the untranslated first exon of both the chick (1) and rat (2) genes is given. Only very short homology regions are seen for these exons (for example the region proximal to the splice junction).

elements are not present in the rat and chick β -actin genes (ref. 22 and T. Kost and S. Hughes, personal communication).

Recently, Davidson *et al.*¹⁹ reviewed the occurrence of conserved sequences in the 5' flanking regions of coordinately regulated genes. The sequence block A reported here is a good candidate for such a sequence because the α -actin gene is one member of a complex set of genes encoding contractile and other muscle-specific proteins which are up-regulated during muscle differentiation^{20,21}. As the skeletal muscle α -actin genes are the only muscle-specific genes which have been completely sequenced to date it is still too early to say whether the conserved sequence block A is present in other muscle-specific genes.

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Note added in proof: Recently, homology with regions B and C has also been found in the 3' untranslated segment of a human skeletal muscle α -actin cDNA (L. Kedes, personal communication).

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Complete nucleotide sequence of an immunoglobulin V_H gene homologue from *Caiman*, a phylogenetically ancient reptile

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Immunoglobulin variable (V) gene regions^{1–10} typify extensive multigenic families in terms of overall size, chromosomal arrangement and presence of large numbers of apparent pseudogenes. A unique mechanism of somatic reorganization involving recombination of V_H , D and J_H or V_L and J_L segments accompanies the differentiation of lymphoid cells^{5,6,11,12} and together with somatic mutation^{6,13,14} and other types of recombination^{15,16} accounts for V-region diversity. Although these processes have been well characterized in higher mammals, little is known concerning their origin and diversification during phylogenetic time. Previously, we described the blot-hybridization characteristics of murine V_H probes with restriction enzyme-digested genomic DNA isolated from several phylogenetically critical species, including *Caiman crocodylus*, a modern representative of an ancient reptilian subclass¹⁷. Here we have used a murine probe, *S107V*, to select homologous clones from a library of *Caiman* genomic DNA constructed in a λ bacteriophage. The complete nucleotide sequence of a *Caiman* gene homologous to the murine V_H gene and its adjacent 5' and 3' region is described. Comparison of the sequence with mammalian prototypes shows evidence of considerable organizational and structural homology extending outside the presumed V_H -coding region and including elements believed to be involved in somatic recombination. Inferences about the evolution of this multigenic family can now be extended to the level of phylogenetic class.

Previously described hybridization–wash conditions¹⁷ were used to screen a *Caiman* genomic DNA library constructed in λ 47.1 (ref. 18) using *S107V*, a murine V_H gene probe⁵ representative of the T15 family of phosphorylcholine-binding antibodies. Multiple positive phage clones were identified and isolated. Clone VIIIB, which contains an ~14-kilobase (kb)

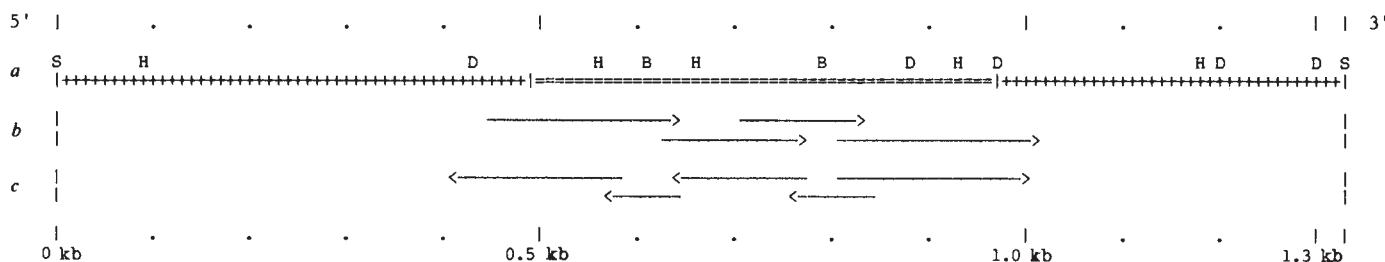
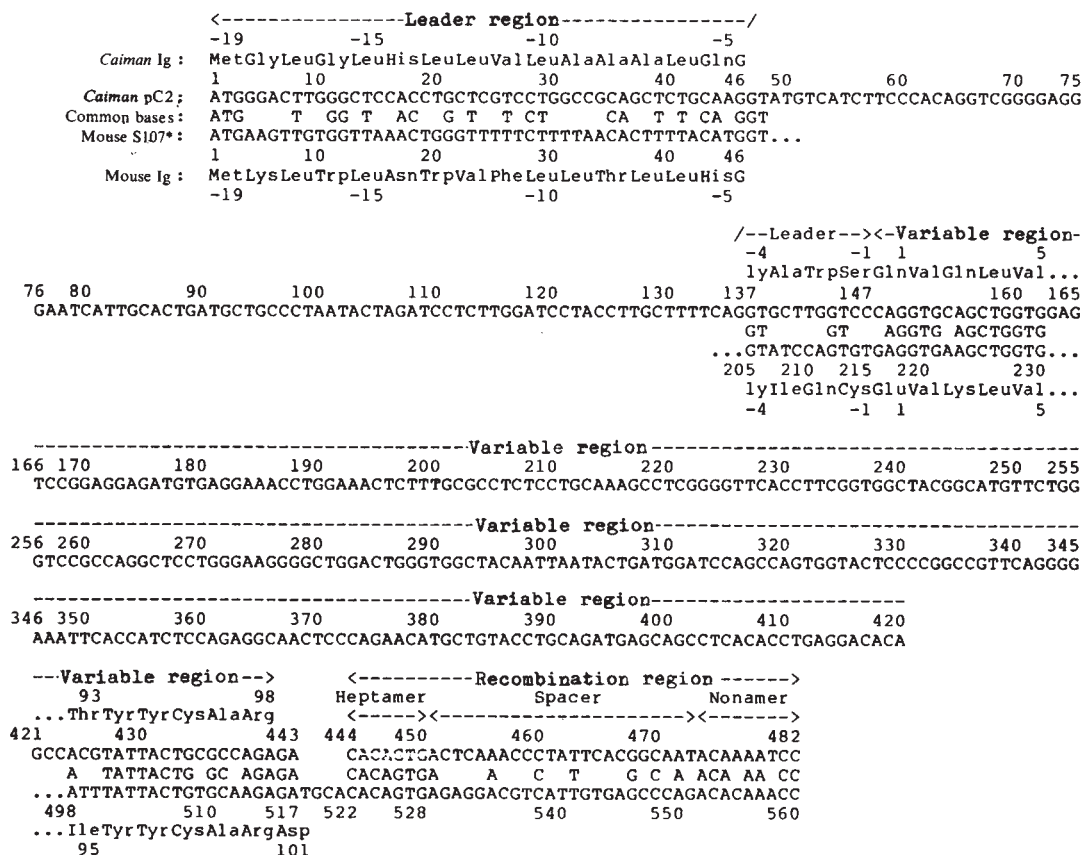


Fig. 1 A partial restriction map of pC3 indicating the principal strategies used to deduce the nucleotide sequence of the presumptive coding and immediately adjacent 5'- and 3'-flanking regions. Partial *Sau3A* digests of high molecular weight (>100 kb) *Caiman* genomic DNA¹⁷ were size-fractionated on a linear 10–40% sucrose gradient. Fractions in the 14–17-kb range were ligated (T4 DNA ligase; NEN) to sucrose density gradient-isolated λ 47.1 arms¹⁸. The efficiency of ligation was verified by agarose electrophoresis and the mixture(s) was packaged directly. Recombinant phage were amplified selectively in *Escherichia coli* Q359.1²⁸. The library was recovered and used to infect *E. coli* KH802, which yields a considerably larger average plaque size than Q359.1. Plaque lifts were screened with nick-translated S107V using a previously described hybridization, reduced stringency wash procedure¹⁷. Screening at successively lower plating densities resulted in the recovery of several different individual recombinant phages (verified by partial restriction mapping) which were purified by polyethylene glycol precipitation followed by two successive bandings in CsCl. In phage VIIIB, hybridization was localized to a single ~1.3-kb *SmaI* fragment, which was recovered preparatively from low melting point agarose and subcloned into *SmaI* site of pLL10 (ref. 19). Parallel blot-hybridization of *SmaI* (*BamHI* or *SstI*)-digested *Caiman* DNA and VIIIB was consistent with the presence of a single germ-line genomic component and suggested that neither the phage nor plasmid cloning procedures resulted in artefactual rearrangement of the germ-line *V_H* homologue. The locations of the restriction sites in pC3 cleaved by *BamHI* (B), *HinfI* (H) or *DdeI* (D) relative to the *SmaI* (S) sites in pLL10 are indicated in *a*. The primary strategy used to determine the nucleotide sequence is indicated in *b* and *c* and used both 5'-end labelling with polynucleotide kinase and 3' fill-in with Klenow fragment of *E. coli* DNA polymerase I (New England Biolabs)¹⁹. The sequence of pC3 presented in Figs 2 and 3 is indicated by = and was determined and verified on both + (*b*) and – (*c*) strands by a minor modification of the partial chemical cleavage method²⁹. 5' Ends are indicated.

Fig. 2 Germ-line sequence of a 482-nucleotide segment of *Caiman* pC3 containing the putative leader, variable and recombination regions. Selected *Caiman* intervals are aligned by metric analysis^{20–22} with the homologous intervals of the germ-line sequence^{5,14} of murine immunoglobulin S107* as follows: *Caiman* 1–46 and mouse 1–46, *Caiman* 137–162 and mouse 205–230, *Caiman* 424–443 and mouse 498–517, and *Caiman* 444–482 and mouse 522–560. S107* refers to the juxtaposing of the germ-line sequence of the *V_H* coding and 3'-flanking regions of S107 described in ref. 5 and the T15 *V_H* leader region described in ref. 14; the intervening sequence of the murine gene is not presented. The 'common bases' line echoes identical homologous nucleotides. The corresponding amino acid sequences of the putative *Caiman* and known mouse immunoglobulins (Ig) are also indicated. By analogy with the murine leader region (mouse 1–46 plus 205–215), the homologous *Caiman* leader region (*Caiman* 1–46 plus 137–147) would encode a transient 19-residue signal peptide. The 5' *Caiman* leader region is interrupted by a 90-nucleotide intervening sequence (*Caiman* 47–136). Based on a Monte Carlo procedure²² that permutes the order of the bases within each interval but keeps the number of each type of base constant, the probability that *Caiman* 1–46 and mouse 1–46 would differ by the observed 25 base changes (or less) by chance alone is less than 1/640. Metric comparison of the *Caiman* and murine variable regions is shown in Fig. 3. Mouse 516–518 encodes Asp 101, which has no counterpart in the *Caiman* sequence. The 3'-recombination regions (*Caiman* 444–482 and mouse 522–560) each begin with the same heptamer (CACAGTG), contain a 23-nucleotide spacer, and end with similar nonamers (ACAXAAXCC).



genomic insert, was characterized further by restriction digestion and blot-hybridization. Based on these analyses, clone VIIIB evidently contains only a single region that hybridizes with the heterologous V_H probe. Note, however, that other clones containing equivalent-sized inserts seem to contain linked gene pairs, as has been noted in similar analyses of human and murine germ-line V_H gene arrangements^{4,6-10}. A ~1.3-kb, hybridizing *Sma*I restriction fragment was subcloned into the *Sma*I site of pLL10 (ref. 19). The subclone is referred to as pC3 and its partial restriction map is illustrated in Fig. 1a. When the pC3 probe was nick-translated and hybridized at reduced stringency to restriction enzyme-digested murine, BALB/c and *Caiman* DNA, multiple hybridizing components were observed which were also detected with the *S107V* probe (not illustrated).

The strategy used to determine the complete DNA sequence of pC3 from the assigned initiation codon to the 3'-flanking region is illustrated in Fig. 1b,c. Computer-assisted analysis of the sequence similarity of the *Caiman* and murine V_H sequences

at the nucleotide level, and indirectly at the amino acid level, was carried out by the metric analysis algorithms²⁰⁻²² recently used in the comparison of influenza neuraminidase genes²³. These algorithms identify nucleotide insertion/deletion events without reading frame bias. Figure 2 illustrates the complete nucleotide sequence of the V_H -coding and flanking regions of pC3, which are aligned with selected (see Fig. 2 legend) homologous segments of a murine prototype.

A significant degree of nucleotide sequence similarity between the two sequences is apparent and the general organizational features of the mammalian 5'-flanking region are conserved. Specifically, a 19-codon leader region extends from the ATG initiation codon (assigned as -19) and splits codon -4, which exhibits splice donor characteristics. A splice acceptor consensus sequence is located ~90 nucleotides downstream and brings the ATG initiation codon into the translational reading phase of the V_H -coding sequence. The hydrophobic character of the peptide encoded by the leader region and the size of the intervening sequence in pC3 are characteristic of



Fig. 3 Alignment by metric analysis²⁰⁻²² of the DNA sequences of the putative variable region (*Caiman* 148-441) of pC3 (Fig. 2) and the homologous variable region⁵ (mouse 216-515) of *S107*†. *S107*† refers to the sequence of the coding region of *S107* described in ref. 5 minus the 3' sequence GAT (Asp), which is absent from the corresponding sequence of pC3. Two nucleotides (or residues) are said to be homologous if they occupy the same alignment position. The distance between these sequences is 101 base changes, which consists of 205 identical homologous nucleotides (identities) scored at 0 base changes each, 89 non-identical homologous nucleotides (mutations) scored at 1 base change each, and 6 nucleotides aligned with null elements (insertion/deletion positions) scored²² at 2 base changes each. The 'common bases' line echoes the 205 identical homologous nucleotides. The 'alignments' line shows the notation '15 :::::' over nine alignment positions of the *Caiman* sequence in order to indicate that *Caiman* 306-310 can be aligned with mouse 375-383 in 15 equivalent ways by rearranging the four null elements (-) over these nine positions. It also shows a dash for each of the 291 alignment positions present in all 15 metric alignments of these two sequences. The 'codon phase' line indicates when the two nucleotide sequences are in or out of phase in the single metric alignment shown. Codons can code for homologous amino acid residues only when they are in phase. Mouse Ig, amino acid sequence of the major part of the V_H domain known to be encoded by murine gene *S107*. *Caiman* Ig, the amino acid sequence of the major part of the V_H domain that may be encoded by *Caiman* pC3. The 'common acids' line echoes identical homologous amino acid residues. The 'domain regions' line shows the framework regions (FR) and complementarity-determining regions (CDR) of the immunoglobulin V_H domain as defined by Kabat *et al.*²⁶. The 'β-strands' line shows the locations observed by X-ray crystallography^{24,25} for all four strands of the outer β-pleated sheet and four of the five strands of the inner sheet of the V_H domain of mouse immunoglobulin M603, a protein closely related^{5,14} to immunoglobulin *S107*.

V-region genes. The pC3 sequence lacks the unusual extended region containing adjacent repeats of the dinucleotide dTdG present in the intervening sequence region of the T15 V_H gene¹⁴.

A high degree of nucleotide sequence similarity between the *Caiman* and *S107* genes is apparent in the variable region, which follows the leader region in the same translational reading frame (Fig. 3). The *Caiman* variable region contains 98 codons, which is 3 codons shorter than the *S107* region⁵ but is similar in length to other murine V_H genes^{6,9,11,12}. The metric alignment illustrates that six null elements must be inserted into the *Caiman* sequence to maximize the nucleotide sequence similarity. The inserted null elements are clustered in nucleotides 369–397 in the second complementarity determining region (CDR). This functionally important region of the *S107* antibody includes the antigen-binding residues Arg 52, Lys 54 and Glu 61 and connects two strands of the inner β -pleated sheet^{24,25}. The *Caiman* and murine sequences shown in Fig. 3 contain 70% identical nucleotides and 56% identical amino acids. When analysed by the CDR and framework regions (FR) shown in Fig. 3, the percentage of nucleotide identities is: FR1, 71%; CDR1, 47%; FR2, 83%; CDR2, 55%; FR3, 74%. The lack of extensive nucleotide sequence similarity in CDR1 and CDR2 suggests that the *Caiman* VIIIB clone does not represent a V_H gene from the T15 family¹³. Rather, the *S107V* probe selected a V_H III homologue having substantial nucleotide similarity in the FR gene segments. The lack of amino acid similarity between the murine V_H CDR1 and CDR2 residues and the corresponding *Caiman* residues further distinguishes these genetic regions.

The *Caiman* V_H sequence is somewhat closer to several human V_H III prototype sequences than to murine prototypes²⁶. Particularly notable is the *Caiman* interval 349–363 (TTCAC-CATCTCCAGA) coding for the pentapeptide Phe-Thr-Ile-Ser-Arg, which is common to all human V_H III sequences. This 15-nucleotide *Caiman* interval is identical to the corresponding sequence of the human V_H III sequence H11¹⁰, but it differs from the murine *S107* sequence by two nucleotides, resulting in two amino acid substitutions. It is possible that the reptilian and human sequences are closer than the murine sequences to a common precursor of these V-region sequences and that the murine sequences contain unique species-specific changes. Such effects would argue against drawing evolutionary generalizations based on comparisons between limited numbers of systematically classified taxa.

In addition to these similarities in the genetic organization and nucleotide and amino acid sequences between *Caiman* and mammalian V_H genes, a typical V_H recombination signal region

follows the presumed V_H coding region. Specifically, the heptamer CACAGTG is followed by a 23-nucleotide spacer and the nonamer ACAAATCC, which resembles the dA+dC-rich mammalian nonamers (defined by the



consensus sequence)^{6,7,9,10,13}. This heptamer/spacer/nonamer pattern of recombination elements also is present following V_L genes, which contain a shorter ~11-nucleotide spacer. The mechanism of somatic reorganization that presently operates to join the V , D and J genes must have evolved before the separation of V_H and V_L gene families. Based on our amino acid sequence data from *Heterodontus*²⁷, functional immunoglobulin genes are present in the phylogenetically early elasmobranchs.

The *Caiman* sequence is the first complete sequence of an immunoglobulin V gene for a species below the phylogenetic level of the mammals. It preserves basic features found in the mammalian V_H gene family even though the *Caiman* and mammalian lineages may have diverged as long ago as 200–225 Myr. Based on hybridization studies using a few heterologous probes¹⁷ and the single homologous probe, pC3, it seems that the V_H gene family is extensive at the level of phylogenetic development represented by contemporary *Caiman*. As suggested by the presence of a typical V_H recombination signal region in the *Caiman* gene, germ-line diversity may be expanded further by somatic reorganization. Our finding of a high degree of structural homology between V_H gene populations from mammals and a reptile suggests that it may be possible to detect related gene populations in even more distant species. Two such strategies to achieve this goal involve the use of (1) probes similar to pC3, which would be isolated from progressively distant species and (2) short oligodeoxynucleotide probes representing the highly conserved sequences, as noted above. Application of these strategies to phylogenetically critical species may help to elucidate the critical events in the formation, stabilization and diversification of complex multigenic families.

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The world through open eyes

Donald MacKay

The Way the World Is: The Christian Perspective of a Scientist.

By John Polkinghorne. *Triangle*: 1983. Pp. 130. Pbk £1.85.

THIS is a refreshing little book. Its author, a distinguished theoretical physicist, is also the only living fellow of the Royal Society in holy orders, and so is eminently qualified to speak for the many practising scientists who find their profession entirely harmonious with their religious commitment, and are puzzled by the persistent notion that the two are incompatible.

What is refreshing is the clean and straight thinking that John Polkinghorne brings to the issue. As a physicist, he has much to say about the revolution in thinking that has overtaken our classical pictures of the physical world. Things are much less clear cut in the world of matter than they were thought to be. But instead of making this a handwaving excuse for lowering our standards of intellectual integrity in matters of religion, he insists that only scrupulous respect for data — given facts — can lead us to a realistic view of the way the world is, in its spiritual as well as its physical aspects. Not open-minded rationality, but fashion-conscious rationalism — the refusal to give due weight to evidence because of preconceived current ideas of what is “reasonable” — is the enemy here; and on this score Polkinghorne does not hesitate to twist (gently) the tails of some modern theologians (p.ix).

His first three chapters offer an extremely readable introduction to the way our scientific picture of the world is moving. The sense of process, of fresh turns of thought taken almost daily in response to fresh evidence, is well conveyed by examples ranging from cosmology (the big bang, the 3°K radiation) and quantum physics to sociobiology. The recognized need for different scientific levels of description of the reality we actually encounter then introduces the main theme of the book, namely, that we miss the whole point of our existence if we do not take seriously enough the religious dimension of reality. The remaining chapters spell out what taking this seriously has meant for John Polkinghorne. Lucidly, with a light touch, he shows how for him an open-eyed commitment to do justice to all the evidence has led to the conviction that “God [has] acted to make himself known . . . not in the shadowy figures of mythology . . . but in the concrete historical figure of Jesus of Nazareth” (p.110). Non-theologians — and non-physicists for that matter — will be helped throughout by an admirable 10-page glossary of scientific and theological terms, which are identified by asterisks as they crop up in the main text.

From a scientific standpoint the outstanding merit of the book lies in the corrective it offers to popular images of science as a spiritually desiccating career:

Wonder at the structure of the world is an authentic part of the scientist's experience. It is the payoff for all those weary hours of study and all the frustrations and disappointments inescapable in the prosecution of research. I believe that [such experiences] are vital to our understanding of the way the world is [p.16].

Again, Polkinghorne makes clear that reductionist *methodology* does not at all entail reductionist *metaphysics*, even though sometimes “A questing agnosticism may be the best we can manage” about relationships between different levels of description (p.25). He rightly cautions against using the *physical* notion of “complementarity” glibly to “explain” such relationships; though unfortunately he fails to distinguish this from the logical concept of hierarchic complementarity, which some would see as essential to a working integration of knowledge we obtain at different levels.

The theological chapters, though quite technical, are attractively laced with humour and commonsense. At one knotty point “we are back”, says Polkinghorne, “in that ‘astronomical’ area of debate where limited data and unlimited human ingenuity can produce a number of answers

of varying plausibility” (p.57). On the other hand,

Christianity, by focusing its understanding of the world on a particular man, can no more be independent of history than a theoretical physicist can neglect the findings of his experimental colleagues. Some Christians have felt that this makes faith intolerably contingent upon history. I welcome that contingency [p.40].

The idea that in Christian thought man is “an animated body and not an incarnated soul” is usefully spelt out, with the memorably expressed conclusion that “the hope of resurrection is very different from being a fly stuck in the amber of divine thought” (p.92). On the other hand, “One learns pretty soon in science that there are times when problems are ripe for solution and times when they are not”. Detailed speculation in this area, Polkinghorne suggests, is at present a waste of time.

Most of Polkinghorne's own speculative judgements abide fairly by his declared canons. An exception is his slight tendency to follow current fashion in patronising the writers of the New Testament (e.g. on p.86 the Apostle Paul, whose name, oddly enough, is not in the index). But the attractive spirit of the book as a whole is well epitomised in a final quotation, from p.26: “If the study of science teaches one anything, it is that it is unwise to try to lay down beforehand by pure thought what will actually prove to be the case. Reality is often so much more subtle than we imagine”. □

Donald MacKay, a physicist, is Emeritus Professor of Communication and Neuroscience at Keele University.

Signal understanding

P.W. Hawkes

Applications of Optical Fourier Transforms.

Edited by Henry Stark.

Academic: 1982. Pp.560. \$67.50, £44.60.

Optical Information Processing.

By F.T.S. Yu.

Wiley: 1983. Pp.562. £43.50, \$69.75.

THE USE of optical methods to manipulate, sieve, enhance and transmit information is growing so rapidly and attracting so much attention, both academic and industrial, that we must expect a steady flow of textbooks and progress reports during the coming years. The volumes of Yu and Stark are both very respectable texts, with some points in common, though, unlike Yu's book, Stark's is a multi-author collection.

The strengths and weaknesses of the second approach are well-known but Stark has chosen his collaborators carefully and

succeeded in persuading them to follow his guidelines, so that the result is more homogeneous than in many such collections. Apart from Stark's opening essay on the theory and measurement of the optical Fourier transform, each contribution deals with a specific class of applications. Generally speaking, these separate accounts are very informative and readable.

I was particularly pleased to see that new developments have not been allowed to squeeze out pioneering work. So, for instance, the chapter by Almeida and Indebetouw, on pattern recognition via complex spatial filtering, reminds us of the work of Duffieux, Maréchal and Croce, Elias, Linfoot, Tsujiuchi, O'Neill, Vander Lugt and Lohmann among many others, before turning to more recent developments. The other chapters, all by recognized authorities, cover particle identification and counting, hybrid systems, radar, photographic measurements, SAW devices, space-variance, non-linear processing, the human visual system, statistical pattern recognition and finally incoherent processing. Anyone wishing to

master the essentials of one or more of these topics fairly painlessly, or to grasp the relation between them, will find this book most useful.

Optical Information Processing is a more formal textbook covering diffraction theory, information processing and holography, with entire chapters on Yu's speciality, rainbow holography. It is intended as a course book as well as for reference, with problems and a number of references at the end of each chapter. The (rather elementary) introductory chapters on diffraction, partial coherence and recording materials, are followed by more technical accounts of Fourier optics and of coherent and incoherent processing methods including polychromatic processing. The final part of the book covers holography and its applications.

The whole text is lucidly written and is ideal for the audience for whom it was intended — electrical engineers (or physicists) with little knowledge of optics, specializing in signal processing. The pace is manageable but this means that many topics are covered in only a rather elementary way so that the book should really be used as a preparation for more advanced and specialized texts: with this proviso, it can be warmly recommended as a course book. The chapters on holography are of wider interest, however, and can be read by anyone wishing to catch up with some of the newer ideas in this field. □

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Journals' review issue 1983

On October 6th *Nature* will publish the third review supplement devoted to science journals. The two previous supplements (covering journals first published between January 1978 and May 1980, and June 1980 and May 1981) appeared in *Nature* 293, 341–369 (1981) and 299, 491–514 (1982).

Criteria for inclusion of a journal in the 1983 issue are that:

- the first number appeared, or the journal was re-titled, between June 1981 and May 1982;
- it is published at least three times a year;
- the main language used is English.

Broadly, periodicals of professional interest to scientists will be considered for review, with the exception of abstracts' journals. Journals appearing prior to June 1981 but after January 1978, and not covered in the previous issues, will also be considered.

Publishers and societies are invited to submit four sample issues of journals satisfying the above criteria, including the first and most recent numbers, to the Review Editor, *Nature*, 4 Little Essex St, London WC2R 3LF, England (London 836 6633 ext 2570) as soon as possible.

Environment: science in politics

Eric Ashby

Science and the National Environmental Policy Act: Redirecting Policy through Procedural Reform.

By Lynton K. Caldwell.

University of Alabama Press: 1983.

Pp. 178. Hbk \$18.50, £16.50; pbk

\$8.95, £7.95.

THE European Commission is at present trying to impose upon its member States a directive obliging them to require environmental impact assessments to be made before certain major projects such as power stations, reservoirs and airports are built. There is, of course, opposition to the directive.

Among the arguments used to support this opposition is the track record of action under the National Environmental Policy Act, passed by the United States Congress in 1969. Section 102 of the Act sets out the requirements for environmental impact statements (EISs) to be made for projects funded by the Federal Government. One visible consequence of Section 102 is that it has spawned millions of words in thousands of reports. The effect of these words on the environment of the United States is by no means so visible. President Carter tried to have the length of the EISs cut down. President Reagan would shed no tears (and, what is more to the point, would, he thinks, lose few votes) if EISs were quietly phased out. The anti-environment lobby in the United States is more confident than it has been since the 1960s. The Council of Environmental Quality has been emasculated; the Environmental Protection Agency (EPA) is in disarray.

This disenchantment with the National Environmental Policy Act is a natural reaction to the euphoria of the 1970s. But it must not be allowed to suppress a very important side-effect of the Act, namely its influence on science. This is the theme of Lynton Caldwell's book. His argument is that an EIS has to be based on advice from several different kinds of experts — engineers, geologists, chemists, biologists, economists, sociologists, lawyers — all reductionists in their own disciplines. These bits of advice are components for a political decision. But who is to assemble the components? Is the politician competent to do it? It is a problem, as Caldwell says, of the management of information for the public good. The specialists may have been brought together as a team, but they don't offer the politician a plan which has a composite pattern: they offer him what Caldwell describes as "a multidisciplinary montage".

For environmental policy, Caldwell believes, multidisciplinary montage is not

acceptable. The experts must offer the politicians an "aggregative and integrative science". This, he says, is explicit in Section 102 of the Act: it requires advisers to take account of what are commonly called unquantified values. The Act, therefore, was a cause (if not *the* cause) for the emergence of what is now called "environmental science". Specialists in the physical, biological and social sciences were challenged to produce advice which had already taken account of the interactions and conflicts between their various disciplines. This would lead to "a new level of integration of knowledge".

To achieve integration there have to be "integrators", which leads Caldwell to discuss the need for science policy to be "managed". This could become a mine-field of controversy, but before scientists get querulous about Caldwell's thesis (as some of them undoubtedly will), it is necessary to understand what the "management" of science under the National Environmental Policy Act means. It means, if I understand it aright, simply asking the right questions; that is, questions which will enable the politician to make a holistic decision out of reductionist information.

I think Caldwell makes his first point, namely that the need (imposed by Congress) to have an environmental policy has created a demand for a fresh kind of expertise, one that will weigh material benefits against ethical values, living standards against quality of life, present consumption against future deprivation. He quotes the sardonic remark made by the Reverend Ralph Abernethy, about the pride of Americans in having secured geological samples from the Moon: when a little black child asked for bread, government gave her a stone.

Caldwell makes a second point, too, namely that conventional education in science is inimical to the production of this fresh kind of expertise. But he is hopeful (and it is at this point that I begin to doubt whether he has made his point): hopeful because there are, all over the country now, departments of environmental science with professors and graduate courses and research programmes. He hopes, too, that the activities of these departments will do something to restore public confidence in science, shaken, as it is, by the obsessive concentration of scientific resources in the United States upon defence. And above all, he hopes that the National Environmental Policy Act will have helped to lift the level of integration of science into a new paradigm.

This boils down to a simple question: is environmental science a new discipline, as genetics and biochemistry and computer science have become disciplines? Many people, even some who could call themselves practising environmental scientists, would, I believe, say "not yet", or even "no". A second question is this: even if environmental science is an emerging new

discipline, is it going to provide the fresh kind of expertise needed to give advice on environmental policy? I confess to doubts on this matter, and for a simple reason. It is that the experts in making political or administrative decisions are politicians and administrators; they are accountable to the public, and it is arguable whether the process of integration should not be left to them. There is a risk that the boundary between what is rational and objective and disinterested information, on the one hand, and its use for making value-judgements, on the other hand, would become blurred if environmental scientists were to take too much responsibility from politicians. Maybe the fresh kind of expert should not be a scientist.

Caldwell's book is a lucid and important contribution to this question. It is also a timely vindication of the National Environmental Policy Act: a sane assessment of its value, written with great

authority, for Caldwell took part in the preparation of the Act. (He records how an early draft of Section 101 of the Act gave Americans the "right to a healthful environment". But Congress shrunk from such a commitment, and amended it to "should enjoy a healthful environment"! He is resentful — as well he might be — about the casual way the present Administration regards environmental issues. But he has faith that the American people, whose ardour for a Green America was so heartening a few years ago, will recover this ardour, shed, perhaps, of some of its weakening extravagances. And, with a change of scenery and a different backcloth, what Caldwell writes about America could be written about Europe. □

Lord Ashby is a Fellow of Clare College, Cambridge. He was Chairman of the Royal Commission on Environmental Pollution from 1970 to 1973.

Change of parasites

Kenneth S. Warren

Modern Parasitology: A Textbook of Parasitology.

Edited by F.E.G. Cox.

Blackwell Scientific/Blackwell Mosby: 1982. Pp.346. Pbk £12.50, \$23.95.

"PARASITOLOGY" is a relatively new discipline! While all infectious agents (viruses, bacteria, fungi, protozoa, helminths) are parasites, it was not until the period 1914–1940 that study of the protozoa and helminths emerged as a separate subject. The basic distinction was that protozoa and helminths were animal parasites; at one time Nuttall even called viruses and bacteria "vegetable parasites". Yet despite the comparatively recent establishment of "parasitology", it is a field which is sorely in need of modernization.

It is gratifying, therefore, to report that *Modern Parasitology* fulfils the call of Paul Weinstein at a conference in 1980 on "The Current Status and Future of Parasitology" (published by the Josiah Macy Jr. Foundation in 1981) for a new type of parasitology textbook organized by "process" rather than by taxonomy, and taking cognizance of the revolutionary changes in

biology in the past few decades. This book, however, is not the major new textbook that Weinstein envisaged. It is precisely what the editor says it is, a book intended mainly for undergraduates to be used in conjunction with standard textbooks. As such it is an important addition to the literature of parasitology.

While the book contains good summaries of combined protozoan and helminth biochemistry, physiology (the rationale for a separate chapter on nutrition is not clear) and immunology, the chapter on epidemiology is particularly worthy of note. This is the new and exciting melding of epidemiology and mathematical ecology pioneered by Roy Anderson and Robert May. Anderson divides the parasites into micro- (viruses, bacteria, protozoa) and macro- (helminths, arthropods), and emphasizes the enormous epidemiological differences between these two groups of organisms.

Perhaps Weinstein's hoped for "major new textbook" will include all of the parasites, animal and vegetable, micro and macro. If the will and the space are not available, however, it might be better if the very different protozoa and helminths were treated separately as subdisciplines (in a manner analogous to viruses and bacteria) interrelated by the biology of parasitism. In the meantime, *Modern Parasitology* lives up to its title and points to the new directions in this field so important to the well-being of humans and domestic animals.

The book is attractively produced and easy to read. It is also remarkably durable, being reviewed on safari in East Africa and emerging from the ordeal in near pristine condition. [.]

Kenneth S. Warren is Director of Health Sciences at the Rockefeller Foundation, New York. He is a co-editor of Parasitology: A Global Perspective, to be published by Springer-Verlag later this year.

Looking for life

A.G. Cairns-Smith

Cosmochemistry and the Origin of Life.

Edited by Cyril Ponnampetuma.

Reidel: 1983. Pp. 385. Dfl. 145, \$63.

HANDS up those who had known all along that the clouds of Venus would be of concentrated sulphuric acid; or that the surface of Mars would be devoid of organic molecules; or that the satellites of Jupiter would be so individual; or . . . Every space probe to the planets has provided surprises, usually several of them. Expect to be surprised, then, by the chemistry of distant parts of the Universe and remote periods of the Earth's history.

We have here a series of review essays, stemming from a NATO Advanced Study meeting held in 1981 (reported in *Nature* 292, 669; 1981). They are written by a well-chosen group of authors and held together by a faith in "chemical evolution", namely that the Universe progresses in some sense towards life via "the atoms of life", "the molecules of life" etc. So we start with element synthesis and go on to organic molecules in deep space, to the search for life or its precursors in the Solar System — and then to Earth, to the evolution of its atmosphere and hydrosphere, and to its ancient sediments.

In the event the surprises outnumber the confirmations of faith. "Chemical evolution" starts well by preferring "the atoms of life" in stellar nuclear synthesis. But when it comes to making organic molecules (most of which in our Universe are out there, between the stars, in gas clouds, on dust grains and in comets) then you have to be optimistic to see any great preference for "the molecules of life". The relevance, it seems, is more oblique: some of the interstellar organic materials are bound to add their atoms to newly forming planets in immense varieties of proportions and circumstances to provide possibilities for evolving organisms made from carbon, nitrogen and so on, in an immense variety of worlds.

Meteorites and lunar samples tell of conditions in the early Solar System, and it seems almost too good to be true that we also have "time probes" relating to conditions on the early Earth itself — in the form of well-dated rocks, the oldest from about 3,800 million years ago. That is a long way back when you consider that the Earth only started forming some 4,500 Myr ago and may well have been only settled enough for life by about 4,000 Myr. There is quite good fossil evidence for photosynthetic organisms 3,500 Myr ago. Although still a matter of debate ¹³C studies on the 3,800-Myr-old rocks are consistent, at least, with a plant life already well established then. More surely, these most ancient rocks tell of an Earth already settled into cycles of weathering and sedi-

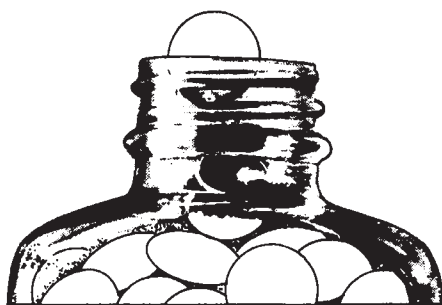
Medical entomology

The third edition of James R. Busvine's *Insects & Hygiene: The Biology and Control of Insect Pests of Medical and Domestic Importance* has been issued in paperback by Chapman & Hall. Price is £12.50.

Vertebrate endocrinology

Cambridge University Press have recently published a second, fully up-dated edition of *Comparative Vertebrate Endocrinology* by P.J. Bentley. The first edition was reviewed in *Nature* 260, 215; 1976. Prices are £32.50, \$49.50 in hardback and £12.50, \$19.95 in paperback.

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REGULATION AND RESTRAINT IN CONTEMPORARY MEDICINE IN THE UK AND USA

Edited by **Dr. Hugh L'Etang**,
Editor of The Practitioner
December 1982 £32.00 224pp
ISBN 0 333 34095 7

Published jointly by the Royal
Society of Medicine and
Macmillan Press Ltd.

Presenting the edited proceedings of an Anglo-American conference, held in October 1981, this book examines in detail the increasing regulatory pressures placed upon medical practice and research both from inside and outside of the profession. These pressures have led to a conflicting array of self-imposed as well as legally imposed constraints, and the contributors discuss here their desirability for both the patient and the profession.

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mentation. They give no indication of an exotic, methane-laden atmosphere: there is a marked decline in enthusiasm for such an atmosphere having ever existed on the Earth. Life started, it seems, under an atmosphere of carbon dioxide, water vapour and nitrogen, with only minor amounts of other constituents. Organic synthesis would still be imaginable, then, in localized regions, but the famous "primordial soup" has become a difficult idea to sustain — particularly since the surface of Mars has so vividly underlined the destructive effects of ultraviolet light.

How on Earth did life start? You will not find an answer to that question in this book; but you will find some of the best evidence available, from front-line exponents, on the geochemical context of biogenesis. And you will find a mind-broadening view of the complexities of the Universe — and an assurance of many surprises ahead. □

A.G. Cairns-Smith is Senior Lecturer in Chemistry at the University of Glasgow, and author of Genetic Takeover and the Mineral Origins of Life (Cambridge University Press, 1982).

Applying colourful antibodies

Brian Anderton

Immunofluorescence Technology: Selected Theoretical and Clinical Aspects.

Edited by G. Wick, K.N. Traill and K. Schauenstein.
Elsevier Biomedical: 1983. Pp.442. Dfl. 140, \$60.

Immunocytochemistry: Practical Applications in Pathology and Biology.

Edited by Julia M. Polak and Susan Van Noorden.
John Wright: 1983. Pp.395. £25, \$45.

A CONSEQUENCE of the competitiveness amongst publishers is the re-working of established topics. These two books are typical examples; although both of them contain new and useful technical information, they include much material which has been adequately covered elsewhere. Indeed, one wonders whether annual practical courses are a suitable basis for such books, as was the case for these two, because of the risk of offending colleagues by omitting their topics since they are not novel.

Immunofluorescence Technology begins well with chapters which live up to the promise of the book's title. These include accounts of preparing defined antigenic substrates consisting of antigens coupled to inert matrices, such as agarose beads, and of how they can be exploited for multiple automated microfluorometric assay of appropriate antibodies. Related micromethods for handling multiple immunofluorescent tests of whole cells are covered subsequently, as are methods for detecting specific antigen-binding cells with fluorescent antigen-coated beads in a rosette-like assay. Relatively recent technological innovations, including the application of lasers as the excitatory source and flow cytometry, are also included.

However, it is apparent from the contributions in the second half of the book — concerned with clinical applications — that these new developments have not yet had a great impact in most diagnostic

laboratories. Although these latter chapters are comprehensive reviews of the application of immunofluorescence microscopy for the diagnosis of conditions such as autoimmune and immune complex diseases, nephropathies and demonstration of tumour-associated antigens, they are all documented elsewhere.

The editors of *Immunocytochemistry* have concentrated on the range of techniques for localizing specific antigens in cells and tissues, both at the light and electron microscope levels. As anyone who has tried his or her hand at immunocytochemistry knows, the choice of fixation method, if any, and detection method is very much dictated by the tissue of interest and the antigen and the antibody, to say nothing of the degree of resolution and histological preservation desired. This book covers all of the possibilities, including semi-thin sections, gold labelling and various methods of double-enzyme labelling with monoclonal antibodies, but it is necessary to spend a certain amount of time sorting out the required information, far too much of which is duplicated. Several of the chapters have an appendix on methodology and, for example, as many as half a dozen describe different variations of indirect immunoperoxidase and PAP methods. Furthermore, there is little uniformity in the layout and extent of details in the protocols — this is a pity and it would have been relatively simple to have had a single appendix giving a comprehensive list of agreed methods.

Greater selectivity exercised by the editors would also have been an improvement. For instance it is questionable whether two chapters on raising antibodies is relevant in such a book, since that is an art all of its own.

These two books will be useful reference sources for the more recent technological developments. However more slender and cheaper versions might have encouraged more owners of Sternberger's *Immunocytochemistry* (rightly praised by Polak and Van Noorden in the introduction to their volume) to purchase them as additional practical aids. □

Brian Anderton is Senior Lecturer in Immunology at St George's Hospital Medical School, London.

Biological Sciences

- ABELE, L.G. (ed.). *Systematics, The Fossil Record, and Biogeography. The Biology of Crustacea*, Vol.1. Pp.319. ISBN 0-12-106401-8. (Academic: 1982.) \$38.50.
- ADAM, W. and CILENTO, G. (eds). *Chemical and Biological Generation of Excited States*. Pp.388. ISBN 0-12-044080-6. (Academic: 1982.) \$59.50.
- ALLEN, T.F.H. and STARR, T.B. *Hierarchy: Perspectives for Ecological Complexity*. Pp.310. ISBN 0-226-01431-2. (University of Chicago Press: 1982.) £19.25.
- ANDREWARTHA, H.G. and BIRCH, L.C. *Selections from The Distribution and Abundance of Animals: With a New Preface*. Pp.275. Hbk ISBN 0-226-02031-2; pbk ISBN 0-226-02032-0. (University of Chicago Press: 1982.) Hbk np; pbk £5.60.
- ANGELL, T. and BALCOMB, K.C. III. *Marine Birds and Mammals of Puget Sound*. Pp.145. Pbk ISBN 0-295-95942-8. (Washington University Press: 1982.) \$14.50.
- ANTOLINI, R., GLIOZZI, A. and GORIO, A. (eds). *Transport in Biomembranes: Model Systems and Reconstitution*. Pp.288. ISBN 0-89004-868-1. (Raven: 1982.) \$39.68.
- ASHER, A. and SPALDING, D.F. (eds). *List of Strains 1982: Culture Centre of Algae and Protozoa*. Pp.82. Pbk ISBN 0-904282-60-0. (Natural Environment Research Council: 1982.) Np.
- BAERTSCH, A.J. and DREIFUSS, J.J. (eds). *Neuroendocrinology of Vasopressin, Corticotiberin and Opiomelanocortins*. Pp.368. ISBN 0-12-072440-5. (Academic: 1982.) £18. \$33.
- BAGSHAW, C.R. *Muscle Contraction*. Pp.79. Pbk ISBN 0-412-13450-0. (Chapman & Hall: 1982.) £2.75.
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- SWETS, J.A. and PICKETT, R.M. Evaluation of Diagnostic Systems: Methods from Signal Detection Theory. Pp.253. ISBN 0-12-679080-9. (Academic: 1982.) £19.20, \$29.
- THOMAS, M.V. Techniques in Calcium Research. Pp.214. ISBN 0-12-688680-6. (Academic: 1982.) £15, \$26.50.
- THOMPSON, D.O. and CHIMENTI, D.E. (eds). Review of Progress in Quantitative Nondestructive Evaluation, Vol. 1. Pp.817. ISBN 0-306-41024-9. (Plenum: 1982.) \$95.
- VINCE, J. Old Farms: An Illustrated Guide. Pp.160. Pbk ISBN 0-7195-3978-1. (John Murray, London: 1982.) £6.95.
- WENNER, L.M. The Environmental Decade in Court. Pp.211. ISBN 0-253-31957-9. (Indiana University Press: 1982.) £13.50 (UK), \$22.50 (US), \$28.13 (elsewhere).

Appointments

HRH the Duke of Edinburgh has been admitted as an honorary member of the Linnean Society of London. Amongst those who already exercise honorary membership are **HM the Queen Mother**, **HM King Carl Gustav of Sweden**, and **HM the Emperor Hirohito of Japan**.

The following have been elected Fellows of the Royal Society of Edinburgh: **Dr R.L.P. Adams** (University of Glasgow, UK); **Dr W. Banks** (The Hannah Research Institute, UK); **Dr A.D.S. Barr** (University of Dundee, UK); **Dr D.J.H. Brock** (University of Edinburgh, UK); **Professor C.M. Brown** (Heriot-Watt University, UK); **Dr A.M. Campbell** (University of Glasgow, UK); **Professor J.H. Collins** (University of Edinburgh, UK); **Dr A.D.D. Craik** (University of St Andrews, UK); **Dr R.M. Cresswell** (The Wellcome Foundation, UK); **Dr G.E. Davie** (University of Edinburgh, UK); **Dr D.A. Ede** (University of Glasgow, UK); **Dr A.R. Forrester** (University of Aberdeen, UK); **Dr B.M.J. Gordon** (Musical Director and Conductor of the Haddo House Choral and Operatic Society, UK); **Dr C.A. Greated** (University of Edinburgh, UK); **Dr V.R.S. Hutton** (University of Edinburgh, UK); **Mr N. MacCaig** (University of Stirling, UK); **Dr R.G.L. McCrone** (Scottish Economic Planning Department, UK); **Professor R. McL. Mackie** (University of Glasgow, UK); **Professor M.C. MacNaughton** (University of Glasgow, UK); **Dr W.B. Martin** (Moredun Research Institute, UK); **Professor W.R.A. Muntz** (University of Stirling, UK); **Professor M. Peaker** (Hannah Research Institute and the University of Glasgow, UK); **Dr J.D. Pitts** (Beatson Institute for Cancer Research, UK); **Dr W.D.I. Rolfe** (University of Glasgow, UK); **Dr P. Tothill** (University of Edinburgh, UK); **Dr A. Truman** (Heriot-Watt University, UK); **Professor P.G. Walsh** (University of Glasgow, UK); **Dr P.C. Wilkinson** (University of Glasgow, UK); **Professor J.H. Williamson** (Heriot-Watt University, UK); **Professor N.G. Wright** (University of Glasgow, UK). This extends

Correction

The new chief medical officer of the British Department of Health and Social Security will be **Professor Donald Acheson**, currently professor of epidemiology and director of the MRC Environmental Epidemiology Unit at the University of Southampton, and not Sir Henry Yellowlees, as was previously announced. Professor Acheson is to replace Sir Henry Yellowlees who has held the post since 1973.

the list of fellows previously announced.

Miscellaneous

As part of the expansion of Biogen's clinical programme aimed at developing gamma interferon, **Dr Seth Rudnick** has joined the company as vice president of medical research for Biogen Research Corporation. Dr Rudnick, formerly director of interferon clinical research at Schering Corporation, will direct the clinical programme worldwide. Biogen is currently completing gamma interferon preclinical trials in Europe under the supervision of Dr Adrian Dawson, who will continue to supervise the clinical trials. Biogen will supply the interferon for the European trials, and also expects to provide gamma interferon on a commercial basis in the future. This contrasts with the programme that is being carried out by Biogen in Japan, for which the interferon is supplied by Shionogi and Company.

Awards

The international Cristoforo Colombo communications award was created by the city of Genoa to honour the name and the memory of Cristoforo Colombo. The prize is intended for the most distinguished contribution to the advancement of communications of either technical, scientific or social significance. For the purpose of making the award, the field of communications has been divided into seven sections: land-communications, telecommunications, communications theory, sea-communications, the technique of information diffusion, air-communications and space-communications. This year's award will go to the information diffusion section. The prize consists of a gold medal together with 5 million Italian lira. It is indivisible and may be awarded to a single person or, collectively, to a group of people.

Novo Industri have announced that the Novo Biotechnology Award for 1983 will be given to **Professor Kei Arima** for his contribution to microbiology and industrial enzymology. Kei Arima is professor emeritus of Tokyo University, president of the Society of Agricultural Chemistry of Japan and president of the Association of Industrial Fermentation of Japan.

The 1983 Hewlett-Packard Europhysics Prize for research into the physics of the solid state has been awarded by the European Physical Society to **Professor Isaac Silvera**. The work for which the award was made was conducted at the Natuurkundig Laboratorium of Amsterdam University and concerns research into the behaviour of hydrogen under extreme conditions.

Frederick Seitz, president emeritus of the National Academy of Sciences and of the Rockefeller University, has been selected to receive the Vannevar Bush Award by the National Science Board of the USA, the policy-making body of the National Science Foundation. The Vannevar Bush Award acknowledges outstanding contributions in science and technology which have particular significance to the national welfare of the United States of America.

Applications are invited for the Biochemical Analysis prize which is awarded biennially by the German Society for Clinical Chemistry for outstanding and novel work in the field of biochemical analysis or biochemical instrumentation, or for significant contributions to the advancement of experimental biology, particularly that relating to clinical biochemistry. Applicants for the DM 10,000 prize, which is donated by Boehringer Mannheim, should submit their papers on one of the above topics (either published or accepted for publication between 1 October 1981 and 30 September 1983) before 15 November 1983 to Professor I. Trautschold, Secretary of the Biochemical Analysis prize, Medizinische Hochschule Hannover, Konstanty-Gutschow-Strasse 8, 3,000 Hannover 61, Federal Republic of Germany.

The British Medical Research Council invites applications for travelling and French exchange fellowships to be taken up in the autumn of 1984 for a period of between three months and one year. Applicants must have been resident in the UK, the Channel Islands or the Isle of Man throughout the three years preceding the application date, although one Alexander Pigott Werner Memorial Trust fellowship in ophthalmology or otology may also be available, and open to Commonwealth candidates who wish to work in the UK. Medically or dentally qualified candidates should be of National Health Service Registrar or Senior Registrar status (or academic equivalent) with some research experience. Non-clinical scientists would be expected to hold a PhD or DPhil degree and normally to have had at least two years' postdoctoral research experience by the time they hope to take up the award. Applicants holding tenured academic appointments are not eligible for this competition but may apply for support under the Council's project grant scheme.

Nominations are invited for the triennial international award of 500,000 rupees which is given by the Hospital Trust Endowment Scheme of the Medical Research Centre of Bombay for outstanding basic or clinical contributions to the field of oncology. Details should be sent to The Director, Rameshwardas Birla Smarak Kosh, Medical Research Centre, Hospital Avenue, Bombay 400 020, India before 31 March 1984.

Meetings

2-3 June, **International Conference on Polymer Photochemistry**, Berlin (Professor W. Schnabel, Hahn-Meitner-Institut, Bereich Strahlenchemie, PO Box 390128, D-1000 Berlin 39, West Germany).

13-15 June, **Course on Agricultural and Forestry Applications of Remote Sensing**, Washington (Continuing Engineering Education, George Washington University, Washington, DC 20052, USA).

26-29 June, **Conference on Laboratory Concerns in Water Pollution Control**, Denver (Water Pollution Control Federation, 2626 Pennsylvania Avenue, NW, Washington, DC 20037, USA).

27 June-1 July, **WHO Course on Statistical Methods in Cancer Epidemiology**, Lyon, France (Research Training and Liaison Unit, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France).

29 June-2 July, **International Seminar on Cereals Technology**, Cambridge (Mr George Jackson, Royal Agricultural Society of England, Stoneleigh, Kenilworth, Warwickshire CV8 2LZ, UK).

4-7 July, **Annual Meeting of the Society of Chemical Industry — Adapting to the Future**, Dublin (Conference Secretariat, Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK).

11-14 July, **The Ciba Foundation symposium on the Photoperiodic Regulation of Insect and Molluscan Hormones**, London (Dr Ruth Porter, The Ciba Foundation, 41 Portland Place, London W1N 4BN, UK).

17-29 July, **International Seminar on Environmental Impact Assessment**, Aberdeen (Mrs Sandra Ralston, Department of Geography, University of Aberdeen, High Street, Old Aberdeen AB9 2UF, UK).

8-27 August, **International Course on Techniques in Molecular Biology**, Santiago, Chile (Dr Jorge Allende, Casilla 6671, Santiago 7, Chile).

11-16 August, **International Conference on High-Energy Accelerators**, Batavia, USA (Conference Secretary, Fermilab, Mail Station 107, PO Box 500, Batavia, Illinois 60510, USA).

14-19 August, **Solar World Congress**, Perth (Congress Secretariat, Solar Energy Research Institute of Western Australia, 13 Howard Street, PO Box X2275, Perth, Western Australia 6000).

26 August-6 September, **International Ethological Conference**, Brisbane, Australia (Conference Secretary, Animal Behaviour Unit, University of Queensland, St Lucia, Australia 4067).

31 August-2 September, **International Conference on On-Line Surveillance and Monitoring**, London (ICOSM '83, The Conference Secretariat, Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK).

1-2 September, **International Symposium on Organ Transplantation in Diabetics**, The Hague, The Netherlands (The Secretariat, ISOTD/Eurotransplant Meeting 1983, PO Box 9600, 2300 RC Leiden, The Netherlands).

1-3 September, **European Congress of Clinical Gerontology**, Budapest (Congress Bureau Motesz, Budapest, PO Box 32, H-1361 Hungary).

4-9 September, **International Symposium on Bioelectrochemistry and Bioenergetics**, Nottingham, (Professor M.J. Allen, Symposium Secretariat, Industrial and Business Liaison Office, University of Nottingham, University Park, Nottingham NG7 3RD UK).

4-17 September, **Course on Recombinant DNA Technology**, Sfax, Tunisia (Dr F. Ben Hamida, Institut Jaques Monod, Tour 43, 2 Place Jussieu, 75005 Paris, France).

5-9 September, **European Symposium on Organic Chemistry**, Canterbury (Dr John Gibson, The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

5-9 September, **International Conference on Phosphorus Chemistry**, Nice (Professor J.G. Riess, Laboratoire de Chimie Minérale Moléculaire, Université de Nice, Parc Valrose, 06034 Nice, France).

5-9 September, **International Symposium on Protein Metabolism and Nutrition of the European Association for Animal Production**, Clermont-Ferrand, France (4ème SIMNA, INRA — Theix, Laboratoire d'Etude du Métabolisme Azoté, 63110 Beaumont, France).

5-10 September, **International Congress of the European Association for Research and Development in Higher Education, "Higher Education by the Year 2000"**, Frankfurt (EARDHE Organizing Committee, Postfach 111 932, D-6000 Frankfurt/M, Federal Republic of Germany).

6-9 September, **Pasteur Biosciences 83, an International Biotechnology Colloquium**, Paris (Pasteur Biosciences 83, Institut Pasteur, 25-28 rue du Docteur Roux, 75724 Paris Cedex 15, France).

7-9 September, **Symposium on Lactic Acid Bacteria in Foods Genetics, Metabolism and Applications**, Wageningen, The Netherlands (Dr P.M. Klapwijk, Unilever Research Laboratory, PO Box 114, 3130 AC Vlaardingen, The Netherlands).

11-15 September, **Symposium on Models of Enzyme Action**, University of Sussex (Dr John Gibson, The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

12-14 September, **International Conference on High-Power Particle Beams**, San Francisco (Dr Alan Toepfer, Arrangements Chairman, Beams '83 Conference, Physics International Company, 2700 Merced Street, San Leandro, California 94577, USA).

12-16 September, **International Symposium on Isotope Hydrology in Water Resources Development**, Vienna

(International Atomic Energy Agency, PO Box 100, A-1400 Vienna, Austria).

12-15 September, **Priestly Conference on Oxygen and the Conversion of Future Feedstocks**, London (Dr John Gibson, The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

13-16 September, **European Solid State Device Research Conference**, Canterbury (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

19-23 September, **Seminar on the Management of Nuclear Power Plants**, Vienna (International Atomic Energy Agency, PO Box 100, Vienna International Centre, A-1400 Vienna, Austria).

15-17 September, **Congress of the European Society for Artificial Organs**, Bologna (Dr Sergio Stefoni, Department of Nephrology and Dialysis, St Orsola University Hospital, via Massarenti 9, 40138 Bologna, Italy).

18-23 September, **World Energy Conference**, New Delhi (Colonel B.T. Nagrani, Executive Director, 12th Congress of WEC, INC.-WEC Secretariat (New Delhi), The Institution of Engineers (India), Bahadur Shah Zafar Marg, New Delhi 110 002, India).

18-23 September, **International Congress on Hormones and Cancer**, Monte Carlo (Dr S. Iacobelli, Laboratorio di Endocrinologia Molecolare, Università Cattolica del S. Cuore, L. go Gemelli, 8-00168 Roma, Italy).

18-23 September, **World Conference on Oleochemicals**, Montreux, Switzerland (Mr James Lyon American Oil Chemists' Society, 508 S. Sixth Street, Champaign, Illinois 61820, USA).

19-21 September, **International Symposium on Advances in Solid-Liquid Separation**, London (The Conference Secretariat, Society of Chemical Industry, 14-15 Belgrave Square, London SW1X 8PS, UK).

19-22 September, **International Conference on Malaria and Babesiosis**, Annecy, France (Dr Micha Roumiantzeff, Foundation Merieux, 17 Rue Bourgelat, 69002 Lyon, France).

19-23 September, **Physical Chemistry of the Solid State: Applications to Metals and their Compounds**, Paris (Dr C. Troyanowsky, Honorary Secretary, Société de Chimie Physique, 10 rue Vauquelin, F 75231 Paris Cedex 05, France).

21-24 September, **International Chromosome Conference**, Lubeck, FRG (8th International Chromosome Conference, Secretariat, Institut für Pathologie, Medizinische Hochschule Lubeck, Ratzeburger Allee 160, D-2400 Lubeck, Federal Republic of Germany).

26-30 September, **International Conference on Hormones and Cell Regulation**, Ste-Odile, France (Dr J.E. Dumont, IRIBHN, Faculty of Medicine, University of Brussels, Campus C Erasme, 808 route de Lennik, B-1070 Brussels, Belgium).